CHANGING PRECIPITATION PATTERNS AND CARBON CYCLING

IN TEMPERATE FOREST SOILS

by

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Abstract

Both carbon dioxide (CO_2) and methane (CH_4) are important greenhouse gases; their increasing concentrations in the atmosphere since pre-industrial times is the main cause of climate change. Methane uptake in soils is a small but important flux in the global budget, with forest soils representing approximately half of the sink. Soil respiration, the largest carbon flux from land to atmosphere, is another significant component of the global carbon cycle. Litter decomposition also plays important role in terrestrial carbon cycling, contributing to the formation of soil organic matter as well as to ecosystem respiration.

Soil moisture, which is influenced by precipitation, is a major driver of soil CH_4 uptake, CO_2 production, and litter decomposition. Precipitation patterns, including annual amounts, timing, variability, and extremity are expected to change in the future all over the world.

To explore how increasing rainfall amount would affect soil respiration in temperate forest soils, we measured soil CO_2 flux at two sites at the Smithsonian Environmental Research Center (SERC) in Edgewater, Maryland: one was regularly irrigated during the growing seasons, while the other just received natural precipitation. Moisture differences were responsible for the relative differences of CO_2 flux between the two sites, specifically for the higher soil CO_2 flux at the irrigated site. However, when volumetric water content was over 37%, increasing soil moisture decreased soil respiration due to oxygen limitation.

To understand how altered precipitation might affect soil carbon cycle, it is necessary to quantify the partitioning of litter carbon into CO_2, dissolved organic carbon (DOC), and soil organic carbon (SOC) during decomposition. We carried out experiments both in the laboratory and in the field to follow litter carbon under normal precipitation and increased extreme events.

^{13}C labeled tulip poplar leaf litter was put on soils from SERC to trace carbon fates during decomposition. Both intensity and frequency of rainfall events were manipulated, while the total amount was kept constant. Extreme rainfall consistently transported more labile litter carbon to greater depths both in the laboratory and in the field. A lower priming effect and more intense physical transfer of particulate organic matter in extreme rainfall resulted in carbon accumulation at surface soil in the lab. In the field, litter decomposition rate was lower in extreme than control treatment due to temporal drought caused by less frequent rainfall events.

We also examined short-term responses of CO_2 and CH_4 after different rainfall treatments with the same lab setup. A pulse of CO_2 efflux was detected right after each rainfall event in the soil columns with leaf litter and the pulse was higher in extreme than control. While rainfall treatments affected the short-term dynamics of CO_2 flux, they had no effect on cumulative CO_2 during the six months of the experiment. Methane uptake rates did not differ between control and extreme rainfall treatments due to high soil moisture conditions. Both CH_4 uptake and CO_2 production rates in litter treatment were higher than those in no litter treatment soils.

Our results suggest that changes in the amount, intensity and frequency of precipitation could influence temperate forest soil carbon cycling through changes in soil respiration, litter decomposition rate, amount of litter derived carbon transported to deeper horizon, priming effect, and CH_4 uptake. Given the significant responses to changing rainfall patterns, some representation of these findings should be included into ecosystem models to better project response of carbon stocks to a changing climate in temperate forest soils.

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1. Introduction

Precipitation patterns, including annual amounts, timing, variability, and extremity are expected to change in the future all over the world due to climate change (Beier et al., 2012; IPCC, 2013). Increasing temperature leads to increased evaporation and surface drying, thereby increasing intensity and period of drought. Higher temperature also brings more water vapor to the atmosphere, which is 7% more per 1 K warming, resulting in a change of annual rainfall amount and intensity of a single rainfall event (Trenberth, 2011).

Records from observations already indicate a change in precipitation patterns both globally and in North America. Globally averaged precipitation over land increased by about 2% between1900-1998 although regional precipitation exhibits high variation (Huntington, 2006). Historical records show an increase in annual precipitation of +9.5±2mm/decade over the 20th century for Northeastern United States (Hayhoe et al., 2006). Apart from annual amount, changes in extreme rainfall events have also been observed. Statistically significant increasing trends of annual maximum daily precipitation have been detected at global scale, which can be related to increase of globally averaged near-surface temperature (Westra, Alexander, & Zwiers, 2013). In the US, based on the National Weather Service Cooperative Observer Network precipitation data from 1948 to 2010, there is strong evidence for a nationally averaged increasing trend in both frequency and intensity of extreme precipitation events (Higgins & Kousky, 2013; Kunkel et al., 2013).

Global Climate Models are generally used to predict how precipitation patterns will change in the future. For annual rainfall amount, dry areas are projected to become drier and wet areas are projected to become wetter, especially in the mid- to high latitudes (Trenberth, 2011). Future projections from almost all models show consistent increase of precipitation in Northeastern United States (Hayhoe et al., 2006). However, projections for extreme rainfall events are consistent all over the world. Increased frequency and intensity of storms, including thunderstorms, snow blizzard, and tropical cyclones, are observed to be widely occurring globally (Trenberth, 2011), nationally (Wuebbles et al., 2014), and locally in Northeastern United States (Hayhoe et al., 2006).

The changes in precipitation patterns, whether in annual rainfall amount or frequency and intensity of extreme rainfall events, can modify soil water availability in terrestrial ecosystems. Altered soil water availability affects ecosystem processes, e.g. primary production, through its influence on plants and soil biota, which in turn will provide feedback to climate change (Beier et al., 2012). Another important ecosystem process is soil carbon cycling, which connects to atmospheric CO_2 levels.

Increasing greenhouse gas levels in the atmosphere since the Industrial Era is the main driving cause of climate change (IPCC, 2013). One important greenhouse gas is carbon dioxide (CO_2), which increased from 278 ppm in 1750 to 390.5 ppm in 2011 by about 40%. This increase is mainly attributed to fossil fuel burning (Andres, Gregg, Losey, Marland, & Boden, 2011), cement production, and land use change (mainly deforestation) (Foley, Monfreda, Ramankutty, & Zaks, 2007; Foley et al., 2011). About half of the emissions remained in the atmosphere, with the other half removed from the atmosphere by marine and terrestrial ecosystems (IPCC, 2013). CO_2 is removed from the atmosphere by photosynthesis at a rate of 123 ± 8 PgC yr^{-1} (Beer et al., 2010), and the carbon fixed into plants is then released back to atmosphere by autotrophic and heterotrophic respiration.

Soil respiration, including respiration from both roots and soil biota, and decomposition of litter and soil organic matter by microbes, represents the largest carbon flux from land to the atmosphere (B. Bond-Lamberty & Thomson, 2010a; Ben Bond-Lamberty, Wang, & Gower, 2004). At a rate of 68 – 80 PgC yr^{-1} (Raich, 1995; Raich & Schlesinger, 1992), soil respiration is an order of magnitude larger than anthropogenic fossil fuel combustion, which is estimated to be 7.8 ± 0.6 PgC yr^{-1} (IPCC, 2013). The high flux comes from a large carbon pool in litter and soils (1500 to 2400 PgC) (Batjes, 1996), which is larger than vegetation (450-650 PgC) (Prentice & Harrison, 2009) and atmospheric CO_2 (819-839 PgC) (Prather, Holmes, & Hsu, 2012) combined. Given the large pool size of soil C and the high flux to the atmosphere, soil respiration could be an important factor influencing CO_2 levels in the atmosphere which will provide feedback to climate change.

The rate at which CO_2 moves from soil to the atmosphere is controlled by the rate of CO_2 production in the soil (the true rate of soil respiration), several factors that influence the diffusion coefficients, such as soil texture and soil structure, the CO_2 concentration gradient at the soil-atmosphere interface and wind speed that affects the non-diffusive transport of CO_2 through and out of the soil (Raich and Schlesinger, 1992). The true rate of production is sensitive to many biotic and abiotic factors, including soil temperature, soil moisture, soil texture, soil nitrogen and other nutrients availability, pH, substrate quality and quantity, vegetation type and others (Dilustro, Collins, Duncan, & Crawford, 2005; Longdoz, Yernaux, & Aubinet, 2000; Raich & Tufekcioglu, 2000; Ryan, Hubbard, Pongracic, Raison, & McMurtrie, 1996). Among the abiotic factors, soil temperature and soil moisture are the two most important factors influencing soil respiration (Raich & Schlesinger, 1992).

Soil respiration generally increases exponentially with temperature. Based on kinetic theory (Arrhenius, 1889), raising temperature increases metabolic reaction rates, and hence increases soil respiration from both plant roots and microbes. This has led to a speculation that global warming will increase CO_2 flux to the atmosphere, decrease the soil organic matter pool and thus provide a positive feedback to future warming (B. Bond-Lamberty & Thomson, 2010b; Z. Zhou, Xu, Kang, & Jianxin Sun, 2015). The relationship between soil respiration and soil moisture is less straightforward. There is a wide range of soil water content within which soil respiration changes little, but as soil dries there exists a point when respiration decreases because microbial and root activity is inhibited due to osmotic stress and limited substrate diffusion (Moyano, Manzoni, & Chenu, 2013; Schimel, Balser, & Wallenstein, 2007). Respiration is also restricted in very wet soils, because a large portion of pores are filled with water, making O_2 less accessible to microbes and roots (Makiranta, Minkkinen, Hytonen, & Laine, 2008).

Additionally, transient increase of soil moisture in dry soils could cause sudden pulse-like events of rapidly increasing CO_2 efflux, which is dubbed as “Birch effect” (Birch, 1964). Four main hypotheses have been proposed to explain the Birch pulse: (1) drying and rewetting shatters soil aggregates and exposes previously unavailable organic substrates for decomposition (Denef, Six, Bossuyt, Frey, & Elliott, 2001); (2) drying causes an increase in dead microbial biomass, which is rapidly decomposed by new microorganisms and fungi after rewetting (Bottner, 1985); (3) spontaneous rapid increase in microbial biomass and fungal hyphae in response to the availability of water (Scheu & Parkinson, 1994); (4) physical replacements of CO_2 in the soil by water (Huxman et al., 2004).

The size of wetting pulses, or the high CO_2 efflux after a rainfall event, depends on the relative change of soil water content. The more severe the drying (i.e., the longer the period or the higher the drying temperature) and the more water added, the larger the amount of decomposition and mineralization on subsequent wetting (Jarvis et al., 2007). The size of wetting pulses also decreases with the frequency of dry-wet cycles, which may be explained by the decrease in labile organic matter over time or by a shift in bacterial community composition (Werner Borken & Matzner, 2009).

Given the strong non-linear response of soil respiration to changes in soil moisture, the changes in precipitation patterns, both amount and frequency in 21st century could potentially have a large and non-linear impact on this carbon flux. Numerous studies that manipulate rainfall patterns have been carried out in different biomes in recent years (Beier et al., 2012). Most of these experiments are conducted in ecosystems where water is a limiting factor and they generally reported soil respiration increased in response to water addition and decreased when soil experienced drought (L. L. Liu et al., 2016; Vicca et al., 2014b; Z. Wu, Dijkstra, Koch, PeÑUelas, & Hungate, 2011). However, the question how increase of precipitation amount will affect soil respiration in mesic systems, where soil water is not limiting such as temperate forests in Northeastern United States, and how intensity of rainfall events combined with leaf litter will influence short and long-term response of CO_2 flux has been less studied.

Soil respiration is only one way, soil could potentially affect atmospheric greenhouse gas levels in other ways. For instance, through restoring degraded soils and ecosystems (Lal, 2004b), large amount of carbon can be sequestered in soils for long term. Soil organic carbons is more than carbon in the atmosphere and vegetation combined (IPCC, 2013), so potential changes in organic carbon would serve either a source or a sink for CO_2 in the atmosphere. Other than carbon storage, soil organic matter can also provide nutrients to plants, maintain soil fertility, and promote favorable structure (Lal, 2004a).

Soil organic matter is defined as the mixture of recognizable plant and animal parts and material that has been altered to the degree that it no longer contains its original structural organization (Amundson, 2001). It is rather heterogeneous, including everything from last minute's root's exudate to 1000-year old persistent mineral-associated organic matter (Janzen, 2006). Leaf litter decomposition is a key component in the cycling of terrestrial carbon and other elements. Products of decomposition, such as CO_2 and partially decomposed organic matter, connect plant production to both the atmosphere and soils (Rubino et al., 2010).

Most litter detritus is derived from three sources: leaves, fine stems and fine roots. A meta-analysis of global literature data on relative mass of leaf, fine stems and fine roots of different trees showed an average forest tree species would shed 41% of its total annual litter production as leaves, 11% as fine stems and 48% as fine roots (Freschet et al., 2013). Given that leaves decompose approximately 1.5 and 2.8 times faster than fine stems and fine roots, respectively, leaves play a major role in decomposition and short term cycling of organic matter in soil (Freschet et al., 2013).

Traditionally, litter decomposition has been divided into three phases (Bjorn Berg, 2000; B. Berg & Ekbohm, 1991; B. Berg, McClaugherty, & Johansson, 1993; B. Berg & Tamm, 1991). In the early phase, the decomposition of water-soluble substances and unshielded cellulose/hemicellulose is stimulated by high levels of the major nutrients (B. Berg & Lundmark, 1987). When all unshielded hemicellulose is decomposed, only lignin-encrusted hemicellulose and lignin remain. In the second phase, the degradation of lignin dominates the litter decomposition rate (Johansson, Berg, & Meentemeyer, 1995). Finally, in the humus-near stage (phase 3), the lignin level is nearly constant, and the litter decomposition rate is close to zero and the accumulated mass loss reaches its limit value (B. Berg et al., 1996).

Application of novel technologies provided new insights to leaf litter decomposition and organic matter transformation. Using compound-specific isotopic analysis, molecules previously thought to persist in soils (e.g. lignin or plant lipids) have been shown to turn over more rapidly than the bulk of the organic matter (Grandy & Neff, 2008; Marschner et al., 2008). At the same time, other labile compounds, such as sugars, can persist for decades (Schmidt et al., 2011). Humic substances were once thought to comprise large and complex molecules, being the largest and most stable soil organic matter fractions (Piccolo, Zaccheo, & Genevini, 1992). However, these humic substances have never been observed by modern analytic techniques. Instead, smaller and simpler molecular structures of organic matter are found *in situ* (Kleber & Johnson, 2010).

Factors that affect litter decomposition rate include (1) climatic factors such as mean annual temperature (MAT), mean annual precipitation (MAP) (Bjorn Berg, 2000; Moore et al., 1999); (2) litter quality such as nitrogen content (Yavitt & Fahey, 1986), and C:N ratio (B. Berg & Ekbohm, 1991); (3) vegetation and litter types (Prescott, Zabek, Staley, & Kabzems, 2000); (4) geographical variables such as latitude (LAT) (Silver & Miya, 2001). Litter decomposition rate generally decreases with increasing LAT and lignin content but increase with temperature, precipitation (Prescott, 2010). At the global scale MAT was more important than MAP (Zhang, Hui, Luo, & Zhou, 2008). However, water availability influenced by precipitation patterns could play an important role in determining litter decomposition rates at local scale (Couteaux, Bottner, & Berg, 1995). Comparing 18 forests in Canada, Moore et al. (1999) found actual evapotranspiration (AET) to be significantly correlated with litter decomposition rate and MAT combined with MAP explained 72-87% of the variance in litter mass remaining. In a field manipulation experiment Lensing and Wise (2007) showed that variability of rainfall patterns, including timing and intensity, could influence litter decomposition rates even when the total amount remained the same.

Besides directly affecting decomposition process via soil moisture and microbial activity, changing rainfall patterns with longer dry periods and more frequent heavy rainfall events can indirectly influence litter decomposition by affecting litter quantity and quality (A. K. Knapp et al., 2008). Ciais et al. (2005) found a strong reduction of gross primary productivity all over Europe in 2003 due to an exceptional heat wave and water deficiency. Altered rainfall patterns could influence tree phenology and tree species distribution (Condit, Hubbell, & Foster, 1996), leading to shifts in both quality and quantity of litter inputs to soil. During plant growth, increased rainfall variability resulted in litter with a higher C:N ratio in tallgrass prairies (Schuster, 2016). Extreme events could also cause sudden, sometimes dramatic changes in litter inputs, such as large increase in aboveground litterfall after hurricanes or severe storms (Ostertag, Scatena, & Silver, 2003).

One important pathway of litter carbon during decomposition is dissolved organic carbon (DOC). DOC movement through soils is an important mechanism in soil formation and thus a factor affecting distribution and stabilization of soil carbon (Trumbore, 1993).

Dissolved organic carbon (DOC) is often defined as solutes passing filter < 0.70 µm in pore size (Michalzik, Kalbitz, Park, Solinger, & Matzner, 2001) and includes a variety of compounds ranging from simple amino acids to complex high-molecular weight DOC. Microbial degradation of soil organic matter followed by desorption of organic substances from solids and leaching of organic substances from fresh litter are thought to be the most important processes causing the release of DOC (Currie, Aber, McDowell, Boone, & Magill, 1996). Under laboratory conditions, the release of DOC from forest floor generally increases with increasing soil temperature, moisture, leaching frequency and decreasing ionic strength (Michalzik et al., 2001). Using data in 42 field studies from temperate forest ecosystems in the northern hemisphere, Michalzik et al. (2001) estimated that annual transport of DOC from the forest floor into the mineral soil amounted to an average of 17% (range: 6 to 30%) of the annual litter carbon input. Annual fluxes of DOC from the forest floor were positively correlated with mean annual precipitation (Michalzik et al., 2001). Both concentrations and fluxes of DOC peak at the O horizon and decrease from the A horizon to the B horizon (Michalzik et al., 2001).

The sharp decrease of DOC concentrations and fluxes with depth indicates either DOC decomposition or adsorption or both (Klaus Kaiser & Kalbitz, 2012). Qualls and Haines (1992) and Frank Hagedorn, Bruderhofer, Ferrari, and Niklaus (2015) investigated the importance of biodegradation in the removal of dissolved organic carbon and reported low rates of decomposition meaning that the major part of litter carbon was retained in soils *via* sorption. Sorption studies using disturbed samples (K. Kaiser & Zech, 1998) and intact soil columns (Guggenberger & Zech, 1992) showed that DOC retention occurs rapidly. Over two thirds of the added DOC was retained in the subsoil horizons within 15 minutes of addition (K. Kaiser & Zech, 1998). One possible group of sorbents for DOC is clay minerals, which is confirmed by the positive relationship between the organic carbon content and the clay content (Müller & Höper, 2004). Indeed, the strong dependence of DOC sorption on pH, the competition of DOC with specifically binding inorganic anions (e.g., phosphate), and the release of OH^- during the sorption suggest that ligand exchange is the most important process in the sorption of DOC on minerals and soils (Gu, Schmitt, Chen, Liang, & McCarthy, 1994).

Despite high sorption of DOC by soil minerals, fast water movement can decrease sorption and microbial processing of DOC, causing litter derived DOC transported deeper into soil than expected (M. Fröberg et al., 2007; F. Hagedorn, Kaiser, Feyen, & Schleppi, 2000). During heavy storm events, water can directly transfer organic solutes from the forest floor to subsoil and further deep to groundwater (Klaus Kaiser & Guggenberger, 2005).

Besides DOC, the other two import pathways of litter carbon during decomposition are CO_2 and soil organic carbon (SOC). While much is known about how climate, litter quality, and decomposer community influence litter decomposition rates, litter is known about what controls the proportion of litter carbon released as CO_2 versus incorporated into soil organic carbon, which is a critical determinant of long-term net ecosystem C balance from the perspective of carbon sequestration (Prescott, 2010).

Based on recent studies that microbial products are the largest contributor to stable SOC (Mambelli, Bird, Gleixner, Dawson, & Torn, 2011) and that the quantity and strength of organo-mineral bonds are the major control on long-term SOC stabilization (Kogel-Knabner et al., 2008), M. F. Cotrufo, Wallenstein, Boot, Denef, and Paul (2013) proposed a framework called Microbial Efficiency-Matrix Stabilization (MEMS) to synthesize these new findings. The idea is that microbial substrate use efficiency (SUE) controls how much litter is being mineralized to CO_2 vs. how much is incorporated into microbial biomass and different byproducts, the ultimate fate of which is determined by their interaction with the soil matrix.

Substrate use efficiency (SUE), the proportion of substrate that is used for growth versus the proportion that is respired, depends not only on substrate quality (e.g., lignin content, C:N ratio and others) (Lekkerkerk, Lundkvist, Agren, Ekbohm, & Bosatta, 1990), but also on microbial community composition and external environmental factors (Manzoni, Taylor, Richter, Porporato, & Agren, 2012). SUE increases with decreasing lignin concentration (Lekkerkerk et al., 1990). Labile litter components can be utilized more efficiently by microbes, contributing more to SOM formation. Change of rainfall patterns can also influence SUE. Under stressful conditions, like droughts or a sudden change of soil water content caused by storms, microbes use more carbon for survival than growth (Schimel et al., 2007), resulting in less soil organic matter formation.

Two dominant pathways contribute to the formation of soil organic matter during decomposition: a dissolved organic matter microbial pathway, which occurs early in decomposition when labile litter components are decomposed and then interact with minerals to form stable SOC, and a physical transfer of litter particulate matter, which happens at the final stage through its inherent chemical recalcitrance (M. Francesca Cotrufo et al., 2015).

Soil organic matter is protected from decomposition by different mechanisms: (1) selective preservation due to recalcitrance of organic matter; (2) spatial inaccessibility of organic matter against decomposer organisms due to occlusion, hydrophobicity and encapsulation; (3) stabilization by interaction with mineral surfaces and metal ions (Phillip Sollins, Homann, & Caldwell, 1996; P. Sollins, Swanston, & Kramer, 2007). Absence of fresh carbon supply can also promote maintaining the stability of SOC at deep soil layers because microbes lack essential source of energy (Fontaine et al., 2007). Chemical recalcitrance and spatial inaccessibility operate at short to medium time scales (e.g., decades), while soil matrix interactions enhance persistence of SOC over the long term (e.g., centuries) (Kogel-Knabner et al., 2008).

During litter decomposition, three pathways contribute to litter carbon loss: CO_2, DOC, and SOC. Due to the complexity of the soil system, the many possible sources of carbon input and processes of carbon transformations, quantifying litter carbon into these three fates is challenging. The application of ^{13}C isotopes provides a useful tool to follow litter carbon during decomposition and thus improved our understanding of carbon cycling at soil surface and deeper horizons. Using ^{13}C depleted leaf litter, A. Kammer and Hagedorn (2011) found mineralization to be the main pathway of C loss from decomposition, with 31% of added leaf litter being lost this way. Most (88 – 96%) dissolved organic carbon was removed while passing through the top 5 centimeters of the mineral soil, where it might have been stabilized. With ^{13}C labeled leaf litter, Rubino et al. (2010) estimated fractions of litter C lost as input into the soil to be twice as much as the fraction of litter C lost to the atmosphere.

The partitioning of three different pathways of litter carbon during decomposition is expected to change in response to different rainfall patterns, especially with increase of extreme events. However, despite numerous previous and ongoing rainfall manipulation experiments, which mostly focused on rainfall amount increase or decrease (Beier et al., 2012), the connection between rainfall patterns and partitioning of litter carbon during decomposition has never been explored.

Apart from CO_2, another important greenhouse gas is methane (CH_4). Although the absolute quantities of CH_4 emission are much smaller than that of CO_2, CH_4 absorbs infrared radiation more strongly per molecule compared to CO_2: over a 100-year period, the warming potential of 1 kg CH_4 is 28 times greater than that of 1 kg CO_2 (IPCC, 2013). Methane increased by 150%, from 722 ppb in 1750 to 1803 ppb in 2011. Between the mid-1980s and the mid-2000s the atmospheric CH_4 growth rate declined to nearly zero (Dlugokencky et al., 2003; Patra et al., 2011). However, since 2006, CH_4 in the atmosphere started increasing again. It is unclear whether this is a short-term variation or a new regime (Dlugokencky, Nisbet, Fisher, & Lowry, 2011).

The balance between CH_4 sources and sinks determines methane growth rate in the atmosphere. The origin of CH_4 can be classified into three types: biogenic, thermogenic, and pyrogenic. Biogenic emissions are the result of degradation of organic matter by methanogen bacteria under anaerobic conditions. Biogenic sources include natural wetlands, waste, landfills, rice paddies, freshwaters ruminants and termites (Dlugokencky et al., 2009). Thermogenic methane is produced from the slow transformation of organic matter into fossil fuels at geological time scales (natural gas, coal, oil). Pyrogenic methane is created by incomplete combustion of organic matter (biomass and biofuel burning). All three origins of methane may come from both natural and anthropogenic sources. Anthropogenic CH_4 sources are estimated to cover 50% to 65% of the global emissions for the 2000s, including rice paddies agriculture, ruminant animals, sewage and waste, landfills, and extraction, storage, transformation, transportation and use of fossil fuels. (Chhabra, Manjunath, Panigrahy, & Parihar, 2013; IPCC, 2013; X. Y. Yan, Akiyama, Yagi, & Akimoto, 2009).

The two ways atmospheric CH_4 can be removed are oxidation by hydroxyl (OH) and chlorine (Cl) radicals in the troposphere and stratosphere and oxidation in upland soils by methanotroph bacteria, with the former contributing 92% to 96% (Nazaries, Murrell, Millard, Baggs, & Singh, 2013) and the latter contributing the remaining (Dutaur & Verchot, 2007). Methane uptake by upland soils is a small but import component of global methane budget, which could be sensitive to changes in land use and climate. Globally, ecosystem type, climatic zone (boreal vs. temperate vs. tropical), and soil texture strongly control CH_4 uptake in upland soils (Dutaur & Verchot, 2007). Ecosystem type accounts for the largest variation in methane uptake capability, with uptake rates in forest consistently higher than any other ecosystems (Boeckx, VanCleemput, & Villaralvo, 1997). Due to the presence of plant detritus, the surface layer of the forest soil has high organic matter, high microbial biomass, low bulk density, and high porosity. Among all the forests, temperate forests with coarse soils have the highest CH_4 uptake rates (7.5 kg CH_4 ha^{-1} yr^{-1}) (Dutaur & Verchot, 2007).

Temperate forest soils both produce and consume CH_4 (von Fischer & Hedin, 2002). The net flux between soil and atmosphere is determined by the balance between methanogenesis (microbial production in anaerobic conditions) and methanotrophy (microbial consumption in aerobic conditions). Methanogenesis usually occurs in wetland soil and rice paddies where soil is waterlogged (Smith et al., 2003). Methane production can also happen in upland soils, where anaerobic 'microsites' occur inside soil aggregates (Dutaur & Verchot, 2007). In addition to microsites, methane can also be produced in anaerobic conditions in saturated zones that coincide with the water table. Upland trees could transport CH_4 from saturated zones below the water table through the transpiration stream, effectively bypassing the zone of CH_4 oxidation, and then diffuse CH_4 to atmosphere through stems (Megonigal & Guenther, 2008; Pitz, Megonigal, Chang, & Szlavecz, 2018). However, methanotrophy is always the dominant process in temperate forest soils, therefore there is a net uptake of CH_4 from atmosphere (Knief, Lipski, & Dunfield, 2003).

Methanotrophs are of two types: low and high affinity group (Shukla, Pandey, & Mishra, 2013). Low affinity group, which operates at CH_4 concentrations > 40 ppm, consumes CH_4 near source areas in soil before CH_4 escapes into atmosphere (B. K. Singh, Bardgett, Smith, & Reay, 2010). High affinity group contributes 90% of total CH_4 consumption in the soil. These microbes function at CH_4 concentrations close to that of atmosphere (< 12 ppm) (Topp & Pattey, 1997).

Methanotroph activities in forest soils are affected by many factors, such as soil pH (Knief et al., 2003), temperature (B. K. Singh et al., 2010), soil moisture (Einola, Kettunen, & Rintala, 2007), nitrogen availability (J. S. Singh et al., 1997), land use (Menyailo, Hungate, Abraham, & Conrad, 2008), copper concentration (Semrau, DiSpirito, & Yoon, 2010), and leaf litter cover (Cheng et al., 2013). In general, litter layer can decrease diffusion of CH_4 from atmosphere into soils, hence reducing CH_4 uptake (Dong, Scharffe, Lobert, Crutzen, & Sanhueza, 1998; Leitner, Sae-Tun, Kranzinger, Zechmeister-Boltenstern, & Zimmermann, 2016), but this effect depends on physical structure of leaves. For instance, replacement of spruce needles by beech leaves in a temperate forest in Germany retarded CH_4 uptake while the reverse process had the opposite effect (Brumme & Borken, 1999).

Soil moisture is another important factor influencing CH_4 oxidation rates. Moisture affects not only the diffusion of CH_4 and O_2 into the soil but also the microbial activity of the methanotrophs (Schnell & King, 1996). At extreme dry conditions, CH_4 uptake is low because microbial activity is low (Dobbie & Smith, 1996). At high moisture levels, the reduced diffusion of both CH_4 and O_2 causes unfavorable conditions for methanotrophs, resulting in low CH_4 oxidation rate (Boeckx, Van Cleemput, & Meyer, 1998). There exists an optimum moisture level for CH_4 oxidation, which, depending on soil texture and structure (Jay Shankar Singh, 2013), ranges between 10% and 25% (VWC) (Boeckx & VanCleemput, 1996; Park, Brown, & Thomas, 2002; Whalen & Reeburgh, 1996).

Due to tight connection between rainfall and soil moisture, shifts in precipitation patterns may affect the soil CH_4 sink. Ni and Groffman (2018) reported a 77% decrease in CH_4 oxidation rates from 1988 to 2015 in forest soils in locations where precipitation has been increasing. In addition to observations, numerous rainfall manipulation experiments explored the effect of changing rainfall patterns on soil CH_4 oxidation rates. Most rainfall manipulations were either water addition or removal (Billings, Richter, & Yarie, 2000; Blankinship, Brown, Dijkstra, Allwright, & Hungate, 2010; Fest, Hinko-Najera, von Fischer, Livesley, & Arndt, 2017), and generally found that CH_4 uptake rates increase in response to water removal and decrease when soil receives increased precipitation, especially during wet seasons (W. Borken, Brumme, & Xu, 2000; W. Borken, Davidson, Savage, Sundquist, & Steudler, 2006). However, experiments with the primary focus on the increase of extreme rainfall events on CH_4 sink in forest soils, have not been carried out.

As described above, soil water content, which is mainly influenced by changing precipitation patterns, could affect multiple soil carbon cycling processes, including soil respiration, litter decomposition, and soil CH_4 uptake. To advance our understanding of the connection between changing precipitation patterns and soil carbon cycling, which can improve models of C cycling in terrestrial ecosystems, and ultimately predict terrestrial carbon-climate feedbacks more accurately, I addressed the following three main questions in my thesis:

(1) How does increased water input influence soil respiration in a temperate forest where soil water is not a limiting factor?

(2) How does the variability of rainfall, especially the increase of extreme events affect the three major pathways of litter carbon during decomposition?

(3) How does leaf litter and rainfall variability affect soil CH_4 uptake and CO_2 efflux in temperate forest soils?

In laboratory and field experiments I tested the following hypotheses:

(1) increase of precipitation would increase soil respiration;

(2) more litter carbon would be transported into deeper horizons and leach out as DOC as frequency of extreme rainfall events increases;

(3) leaf litter would reduce CH_4 uptake; soil CH_4 oxidation rates would be higher under extreme treatments; extreme rainfall treatment would trigger a greater CO_2 pulse than control but cumulative CO_2 would not be different.

Chapter 2 of my thesis explores the effect of increased precipitation amount on soil respiration in a mature temperate forest at the Smithsonian Environmental Research Center (SERC) in Edgewater, Maryland. To manipulate increased rainfall, water was added to an experimental plot during the growing seasons of 2014-2015. Chapter 3 describes a laboratory experiment to study the effect of changing rainfall patterns, especially the increase of extreme rainfall events, on three pathways of litter carbon, using forest soil collected at SERC. ^{13}C labeled tulip poplar leaves were used to follow the fate of litter carbon. Chapter 4 describes a field experiment with similar setup as Chapter 3, in which leaf litter was put directly on the soil surface and decomposed *in situ.* This experiment allowed us to test if results are consistent in the lab and field. Chapter 5 presents the first study to simultaneously explore the effects of leaf litter and rainfall variability on forest soil CH_4 uptake and CO_2 efflux rates using the same setup as Chapter 3. Rainfall treatments are based on decades of historical precipitation data from nearby weather stations, and were the same in Chapters 3, 4, and 5.

2. The effect of increased precipitation on soil respiration in a temperate forest in Maryland, USA

Abstract

Soil respiration produces a major flux of CO_2 from terrestrial ecosystems to atmosphere and can act as a feedback to climate change. Patterns of precipitation are expected to change in the future, including an increase of precipitation in Northeastern United States. However, the effect of increasing precipitation on soil respiration is not well understood in temperate forest where soil moisture is not limiting. Here we report soil respiration in a temperate forest with irrigation during growing seasons to simulate increasing rainfall in two years. Soil respiration increased exponentially with temperature and there existed a quadratic relationship between soil respiration and soil moisture, with an optimal value of 37%. Change of soil respiration depended not only on increased rainfall amount but also on initial soil moisture: water addition only enhanced soil respiration when initial soil moisture was below the optimum.

2.1 Introduction

Soil respiration, the flux of carbon dioxide from the soil surface to the atmosphere, represents the second-largest terrestrial carbon flux after photosynthesis and can account for 60-90% of total ecosystem respiration (Goulden, Munger, Fan, Daube, & Wofsy, 1996; Longdoz et al., 2000). This high flux, which is an order of magnitude larger than anthropogenic fossil fuel combustion (B. Bond-Lamberty & Thomson, 2010a), comes from a large pool: globally, soils store at least twice as much carbon as in the atmosphere (Tarnocai et al., 2009). Given the large pool size of soil carbon and high flux, soil respiration is therefore considered one of the most significant components of global carbon balance (B. Bond-Lamberty & Thomson, 2010a).

Soil respiration consists of two components: autotrophic and heterotrophic respiration. Autotrophic respiration refers to the respiration by living plant, including plant root and rhizo-microbial respiration while heterotrophic respiration is microbial decomposition of plant litter and soil organic matter, and respiration by soil fauna. Soil respiration is often determined by measurement of CO_2 flux from the soil surface. We will refer to CO_2 efflux rates as being equivalent to soil respiration, but emphasize the soil CO_2 efflux rates, not actual soil respiration rates, are measured.

Many factors could affect soil respiration rates: soil temperature(Raich & Schlesinger, 1992), soil organic matter quantity and quality (Longdoz et al., 2000), root and microbial biomass, root nitrogen content (Ryan et al., 1996), soil texture (Dilustro et al., 2005), and vegetation type (Raich & Tufekcioglu, 2000). Another important abiotic factor is soil moisture. Soil moisture influences soil respiration directly through physiological processes of roots and microorganisms, and indirectly via diffusion of substrates and O_2. The common conceptual relationship states that soil CO_2 efflux is low under dry conditions, reaches the maximal rate in intermediate soil moisture levels, and decreases at high soil moisture content when anaerobic conditions prevail to depress aerobic microbial activity (L. Xu, Baldocchi, & Tang, 2004).

Precipitation is a major driver of soil moisture. Precipitation patterns, including annual amounts, timing, variability, and extremity are expected to change in the future all over the world. Precipitation amount over land increased by about 2% between 1900-1998 if averaged globally despite of high variation of regional precipitation (Huntington, 2006). Future projections from almost all models show consistent increase in precipitation amount in Northeastern United States (Hayhoe et al., 2006).

Numerous precipitation manipulation experiments have been conducted to explore the effect of changing precipitation patterns on soil respiration in several biomes (Beier et al., 2012). Most studies were conducted in ecosystems where water availability is below optimum level (L. L. Liu et al., 2016; Z. Wu, Dijkstra, Koch, Penuelas, & Hungate, 2011), and found changes in soil respiration were positively correlated with the changes in soil moisture. However, the question how increasing precipitation will affect soil respiration in temperate forest in Northeastern United States, where water is not limiting, is not well understood.

To examine the effect of increased precipitation on physiology and growth of trees in a temperate forest, an irrigation experiment simulating increased rainfall amount was set up at the Smithsonian Environmental Research Center in Edgewater, Maryland during growing seasons in years 2014 and 2015 (Figure 2-1). We conducted weekly soil respiration measurements during growing seasons at this site. We hypothesized that (1) water addition simulating increasing rainfall would increase soil respiration and (2) changes in soil respiration would be positively related to changes in soil moisture.

2.2 Methods

2.2.1 Site description

The Smithsonian Environmental Research Center (SERC) is located along the western shore of the Chesapeake Bay in Edgewater, Maryland on the Rhode River estuary (38º53'N, 76º33'W). The major soils at the upland forest are Collington sandy loam (fine-loamy, glauconitic, mesic Typic Hapludult) and Donlonton fine sandy loam (fine-loamy, glauconitic, mesic aquic Hapludults). Parent material in this region is glauconitic marine sediments lying on the Nanjemoy formation (Yesilonis, Szlavecz, Pouyat, Whigham, & Xia, 2016). The mean precipitation is 114.6 cm and the mean annual temperature is 13°C. Our study site is an old forest stand with a mixed history of agriculture and logging. The land was abandoned 150 years ago and today it is dominated by several species of oaks (*Quecus* spp.), American beech (*Fagus grandifolia*), and hickories (*Carya* Spp.). Total carbon content at 0-10 and 10-20 cm is 3.04% and 1.05%, and pH ($CaCl_2$) at 0-10cm and 10-20cm is 4.8 and 4.7, repectively (Yesilonis et al., 2016).

2.2.2 Experimental design

At our old forest site, two adjacent areas that have similar soil properties and tree composition and are more than 100 meters apart were chosen. One area of 380 m^2 received water addition with soaker hoses connected to water tanks to simulate increasing precipitation (IP) during growing seasons in 2014 and 2015, and the other area received ambient precipitation (AP) (Figure 2-1). In 2014, 108 m^3 of water was added to IP plot from Jun 23rd to September 30th. In 2015, 183 m^3 of water was added more frequently from May 7th to September 4th. The water addition amounted to 25% and 42% increase of mean annual rainfall amount in 2014 and 2015, respectively.

2.2.3 Soil respiration measurements

At each forest plot, a 20 x 20 m^2 area was chosen and in May 2014 six PVC soil collars (80 cm^2 in area and 10 cm in height) were permanently installed 5 cm into the soil. The locations of the collars were randomly chosen with the distance between adjacent collars being at least 50 cm. In May 2015, two additional PVC collars were installed in IP plot.

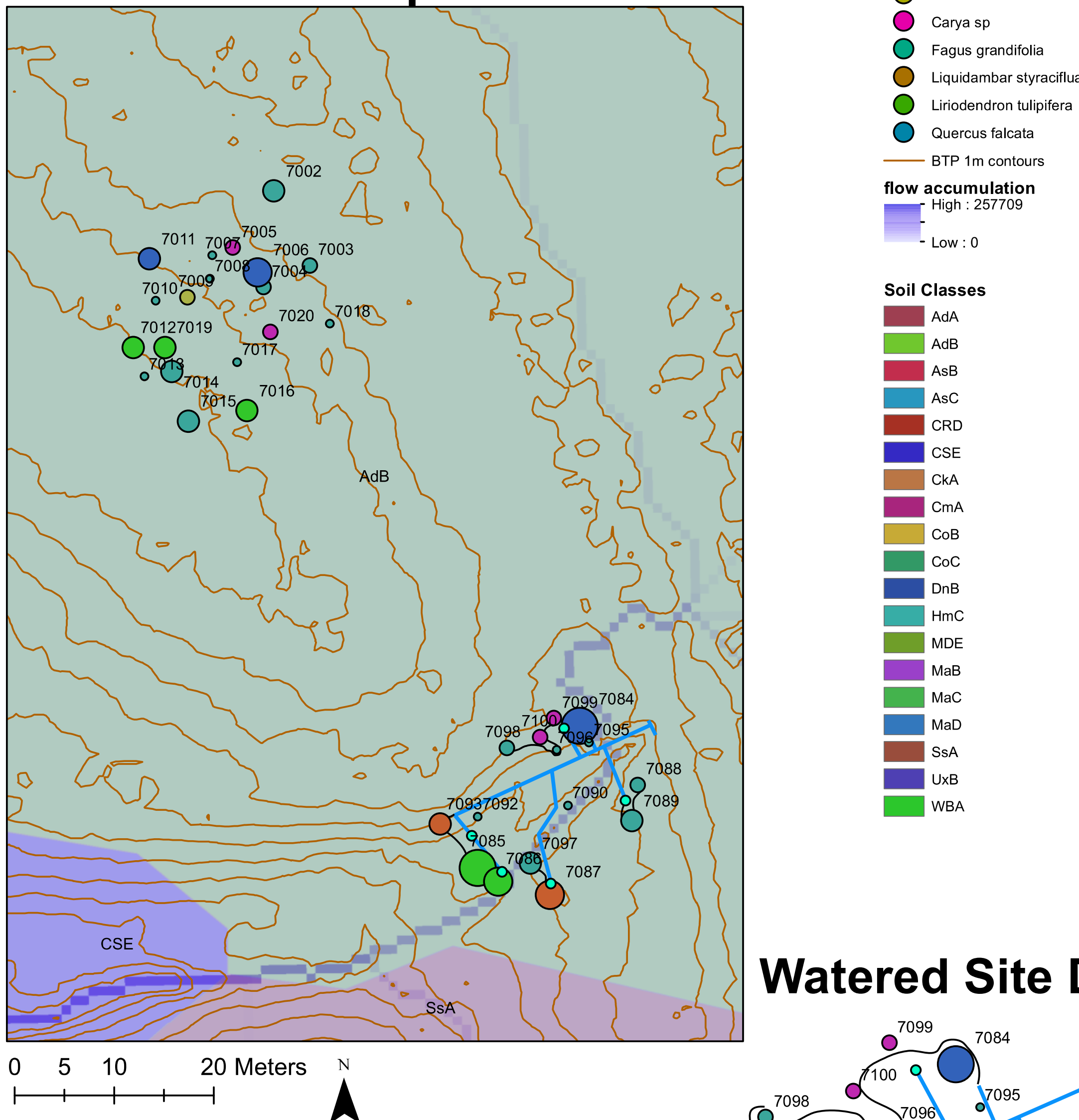
Soil respiration was measured weekly during growing season in 2014 and 2015 and bi-weekly during non-growing seasons from November 2015 to May 2016. A PVC chamber assembled with a CO_2 sensor (GMP 343 Vaisala, Finland) was placed on the collar while measurements were taken. CO_2 concentrations in the headspace are recorded every second for 6 minutes. CO_2 efflux, F, can be calculated as:

$$F = \frac{dC}{dT} \times \frac{V}{S}$$

where F is the gas flux in μ mol·m^{-2} ·h^{-1}, C is the mole concentration in μ mol·m^{-3}, T is time, V is the volume of headspace, and S is the area of the soil surface in the chamber. dC/dT can be approximated as slopes of fitted lines between CO_2 concentration and time. The coefficient of determination (R^2) values of these fitted lines are usually larger than 0.99.

Soil respiration measurements were made between 9:00 am and 3:00 pm local time. Soil temperature at 10 cm depth was also measured with MULTI-THERMO digital thermometer. Volumetric water content (VWC) of the top 10 cm soil layer was measured with NH2 moisture meter coupled with ML3 ThetaProbe (Delta-T Devices) at the same time when the soil respiration measurements were being taken.

Beech Cluster Experiment Site



Watered Site Detail

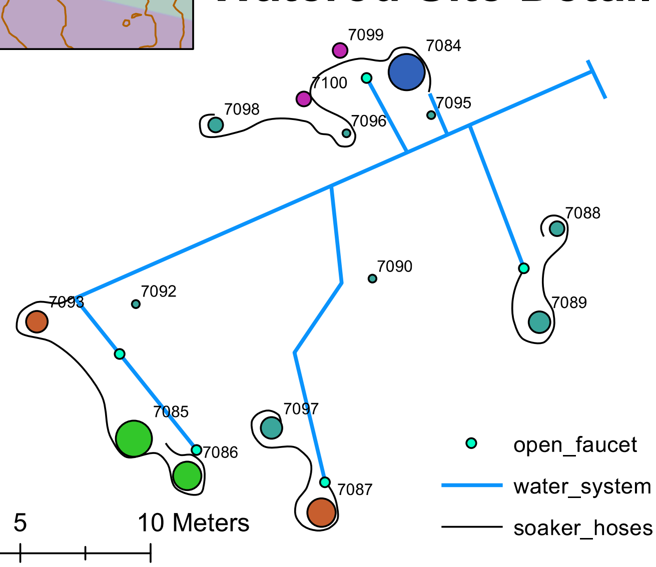


Figure 2-1 The irrigation experiment site map at Smithsonian Environmental Research Center (SERC)

2.2.4 Statistical analysis

All data analyses were carried out using R software version 3.3.3. Soil respiration rate, soil temperature, and volumetric water content were calculated as the means of all the chamber measurements in that plot. Linear mixed models were run to evaluate the effect of extra watering on temperature, volumetric water content, and soil CO_2 efflux. Column number was treated as random effect, date and irrigation treatments were treated as fixed effect. Factors were evaluated in the models in the above order. The above linear mixed models were also run separately in different years and watering seasons to assess the effect of irrigation in different years and in watering vs. non-watering seasons. The effect of year and watering on the differences of VWC and CO_2 efflux in IP vs. AP plots were evaluated by performing two-way ANOVA.

To explore the relationship between soil respiration and soil moisture (VWC), measured respiration rates were fit to soil moisture content using the quadratic function:

$$F = y_0 + bx + ax^2$$

where F is the measured CO_2 flux (mg m^{-2} h^{-1}), x is the volumetric water content (%), and y_0, a, and b are constants. Only soil respiration rates during watering seasons were used to fit the model when temperature was higher than 16°C to rule out a temperature effect on soil respiration. The optimal value was calculated as –b/2a, when the function above has the highest value.

We assessed the temperature sensitivity of CO_2 efflux (F) by fitting data collected both in 2014 and 2015 in watering seasons within each individual treatment to the exponential function:

$$F = ae^{bT}$$

where F is the measured CO_2 flux (mg m^{-2} h^{-1}), T is the soil temperature at 10 cm depth (°C), a is the basal respiration and b is the temperature sensitivity of soil CO_2 efflux. Only respiration rates with a lower VWC than threshold were used to fit the model. Respiratory quotient (Q_{10}) can be calculated as:

$$Q_{10} = e^{10b}$$

A linear regression model was used to explore the relationship between changes in soil respiration (F_{diff}) and changes in VWC (VWC_{diff}) and *VWC* at AP plot using the model below:

$$F_{diff} = a + bVWC_{diff} + cVWC + dVWC^2$$

where a, b, c, and d are constants.

2.3 Results

There were no strong seasonal variations of precipitation in two years (Figure 2-2B). The annual precipitation was 906 and 745 mm in 2014 and 2015 respectively. The monthly mean precipitation ranged from 24 mm (July 2015) to 164 mm (June 2015). Daily mean air temperature varied from -12.5 °C to 29.3 °C, and annual mean air temperature was 12.8 °C and 13.0 °C in 2014 and 2015 respectively (Figure 2-2A).

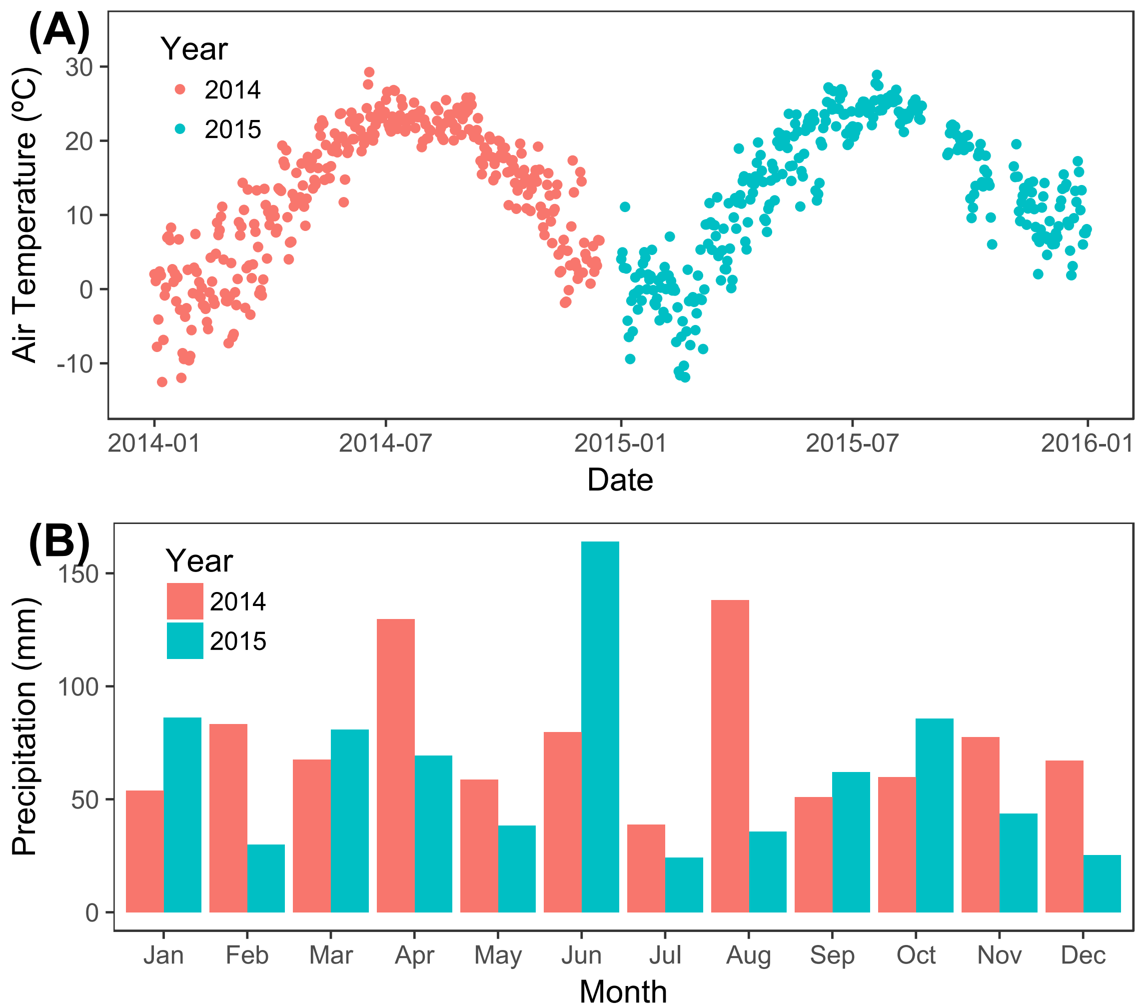


Figure 2-2 Daily mean temperature (A) and monthly rainfall (B) at Smithsonian Environmental Research Center during the experimental period from 2014 to 2015.

No difference in soil temperature was found between AP and IP plots (Figure 2-3, Table 2-1).

Soil VWC was significantly influenced by irrigation treatment. VWC in IP plot was significantly higher than that in AP plot (Figure 2-4, Table 2-1). VWC ranged from 8.4% to 52.6% in AP plot and from 10.5% to 68.4% in IP plot. The IP treatment increased mean VWC from 28.5 ± 9.7% to 36.3 ± 14.5% by 27%. The differences of VWC in AP vs. IP were all significant except in 2015, non-watering season (Table 2-2). The highest difference of VWC was in

2015 watering season, followed by 2014 watering season and then 2016 non-watering season, with 2015 non-watering season coming the last (Figure 2-6).

Table 2-1 Results of mixed effect models testing the effects of precipitation treatments on soil temperature (T), volumetric water content (VWC), and soil CO2 efflux

	Date		Precipitation	
	χ^2	P	χ^2	P
T	2684.7	< 0.001	0.1	0.781
VWC	1386.2	< 0.001	10.8	0.001
CO_2	750.5	< 0.001	9.9	0.002

Table 2-2 Results of mixed effect models testing the effects of precipitation on VWC and soil CO2 flux in different years and watering vs. non-watering seasons separately

		Date		Precipitation	
		χ^2	P	χ^2	P
2014 Watering	VWC	143.3	< 0.001	8.7	0.003
	CO_2	73.0	< 0.001	8.8	0.003
2015 Watering	VWC	646.9	< 0.001	11.9	<0.001
	CO_2	184.1	< 0.001	9.5	0.002
2015 Non-watering	VWC	327.9	< 0.001	2.8	0.095
	CO_2	229.3	< 0.001	9.2	0.002
2016 Non-watering	VWC	160.1	< 0.001	8.2	0.004
	CO_2	50.0	< 0.001	6.0	0.014

Table 2-3 Parameters and p values for fitting difference of soil respiration using difference of VWC (VWC_diff) and VWC at AP plot

	Parameter estimate	P
Intercept	-45.78	0.09
VWC_diff	4.11	< 0.001
VWC	7.81	< 0.001
VWC^2	-0.17	< 0.001

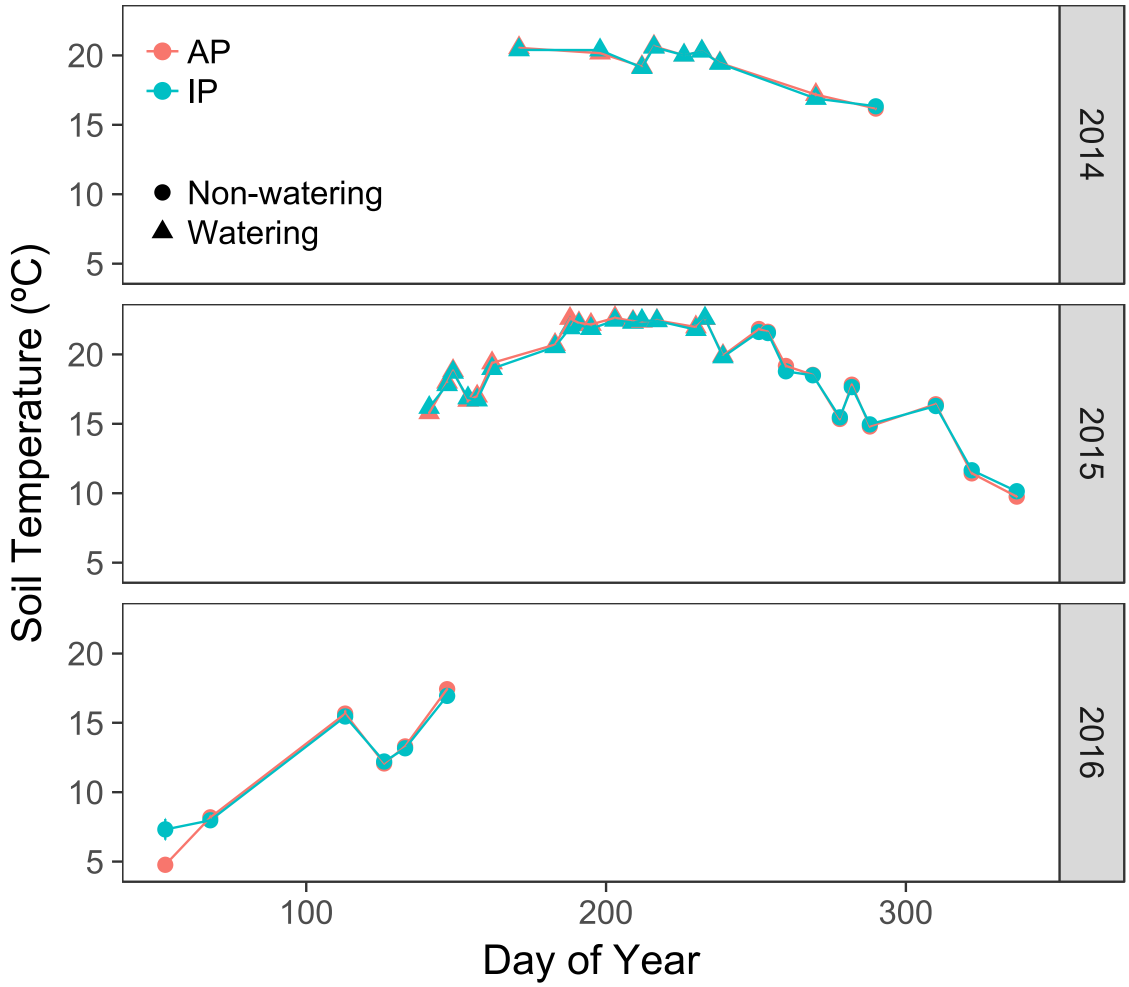


Figure 2-3 Soil temperature at 10 cm depth in year 2014, 2015, and 2016. Error bars represent standard error

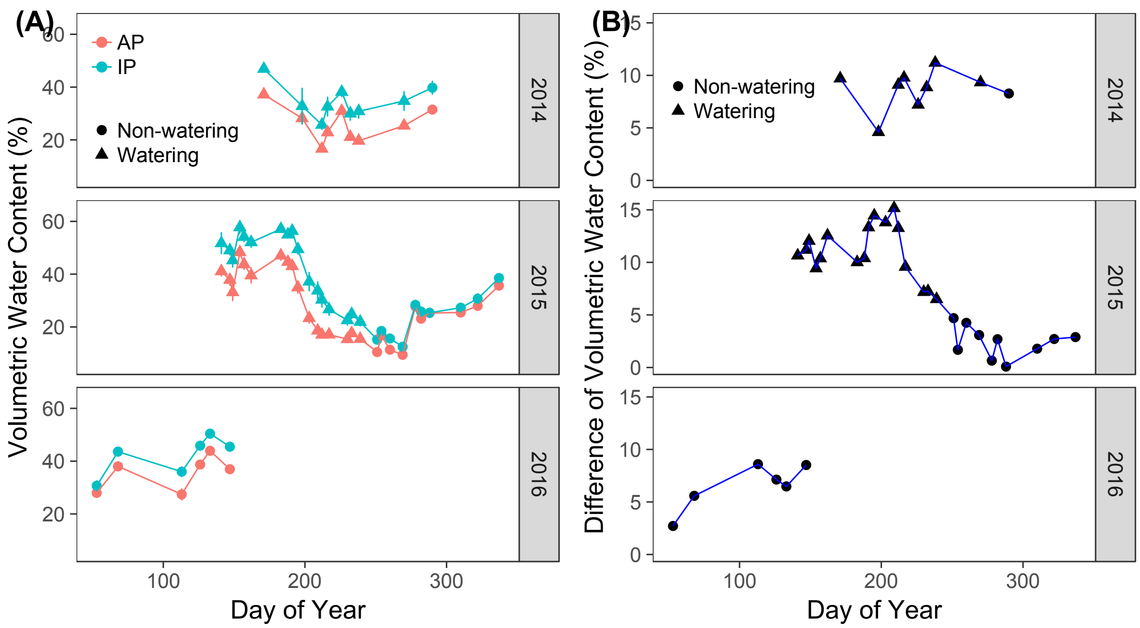


Figure 2-4 Volumetric water content (VWC) (A) and difference of VWC (IP – AP) (B) in different years. Error bars represent standard error

CO_2 efflux was also higher in IP than AP plot (Figure 2-5, Table 2-1). CO_2 efflux ranged from 17.8 to 341.1 CO_2-C mg/m^{2}h^{-1} in AP plot and from 37.6 to 594.5 mg/m^{2}h^{-1} in IP plot. Irrigation treatment increased mean CO_2 efflux from 142.3 ± 78.4 mg/m^{2}h^{-1} to 194.8 ± 128.8 mg/m^{2}h^{-1} by 36.9%. The differences of CO_2 efflux in AP vs. IP were all significant (Table 2-2), even in 2015 non-watering season, when difference of VWC in two sites was not significantly different and the smallest. The difference of CO_2 efflux was the highest in 2014 watering season, followed by 2015 watering season, 2015 non-watering season, and 2016 non-watering season (Figure 2-6).

There existed a quadratic relationship between soil respiration and VWC (R^2 = 0.12), and threshold VWC is 37.0% (Figure 2-7). The temperature sensitivities among years and treatments were not significantly different, so all data points were combined for model fitting. The relationship between soil respiration and soil temperature was exponential (R^2 = 0.49), with a

Q_{10} of 3.64 (Figure 2-8). The parameters estimate from fitting changes in soil respiration using changes in soil moisture and soil moisture at AP plot were all statistically significant except the intercept (Figure 2-9, Table 2-3). The model explained 63% of variance of changes in soil respiration, with difference of VWC having a positive effect and VWC at AP plot having a non-linear effect.

Cumulative CO_2 was 439.1 and 671.8 g C m^{-2} for AP and IP in 2014 (14-06-20 to 14-10-17), and 568.7 and 729.1 g C m^{-2} for AP and IP in 2015 (15-05-21 to 15-10-15). The increase of cumulative CO_2 (IP – AP) compared to AP plot was 53% and 28% in 2014 and 2015, respectively.

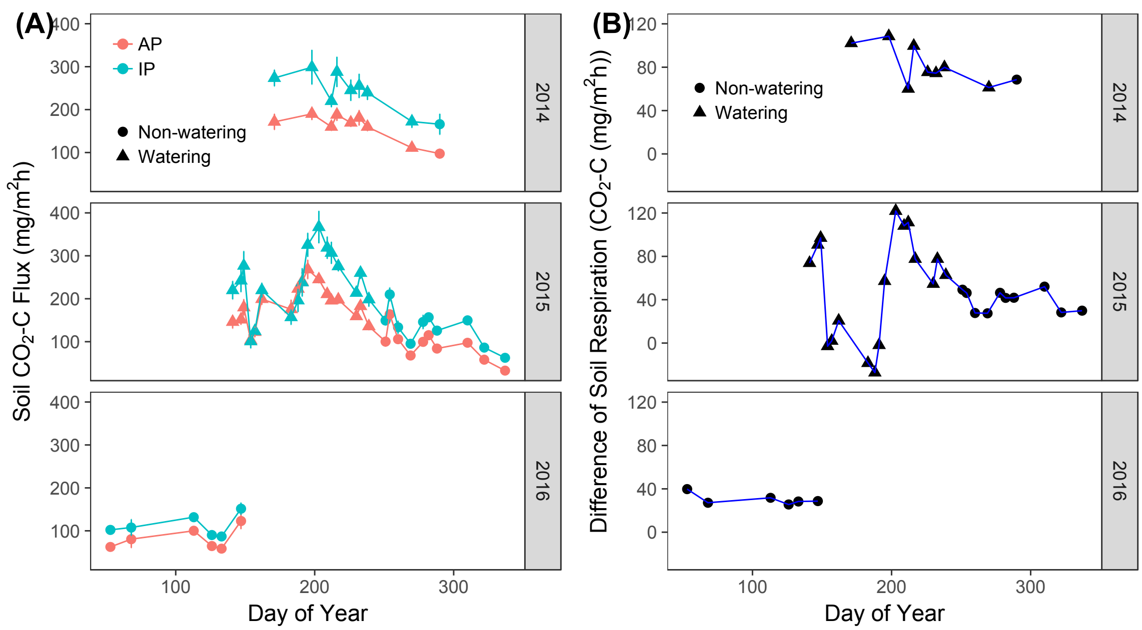


Figure 2-5 Soil respiration (A) and difference in soil respiration (IP – AP) (B) in different years. Error bars represent standard error

2.4 Discussions

Based on kinetic theory (Arrhenius, 1889), it is widely accepted that soil respiration increases with temperature. Consistent with this expectation, we found an exponential relationship between soil respiration and temperature (Figure 2-8). Higher temperature would usually lead to a higher CO_2 production in soils because of a higher metabolic activity by both microbes and roots (Bergner, Johnstone, & Treseder, 2004; Z. Wu, Dijkstra, Koch, Penuelas, et al., 2011). Higher temperature could also indirectly influence soil respiration rate by increasing gas diffusion process (Troeh, Jabro, & Kirkham, 1982), which is the main transport mechanism for CO_2 to be released back to the atmosphere. Most biogeochemical models represent the relationship between soil respiration and temperature using a fixed Q_{10} (T. Zhou, Shi, Hui, & Luo, 2009), which is defined as the factor by which soil respiration increases by a 10 °C increase in soil temperature. The Q_{10} at our site is 3.64, which is within the range of Q_{10} values from 1 to 12 (Hamdi, Moyano, Sall, Bernoux, & Chevallier, 2013; Meyer, Welp, & Amelung, 2018).

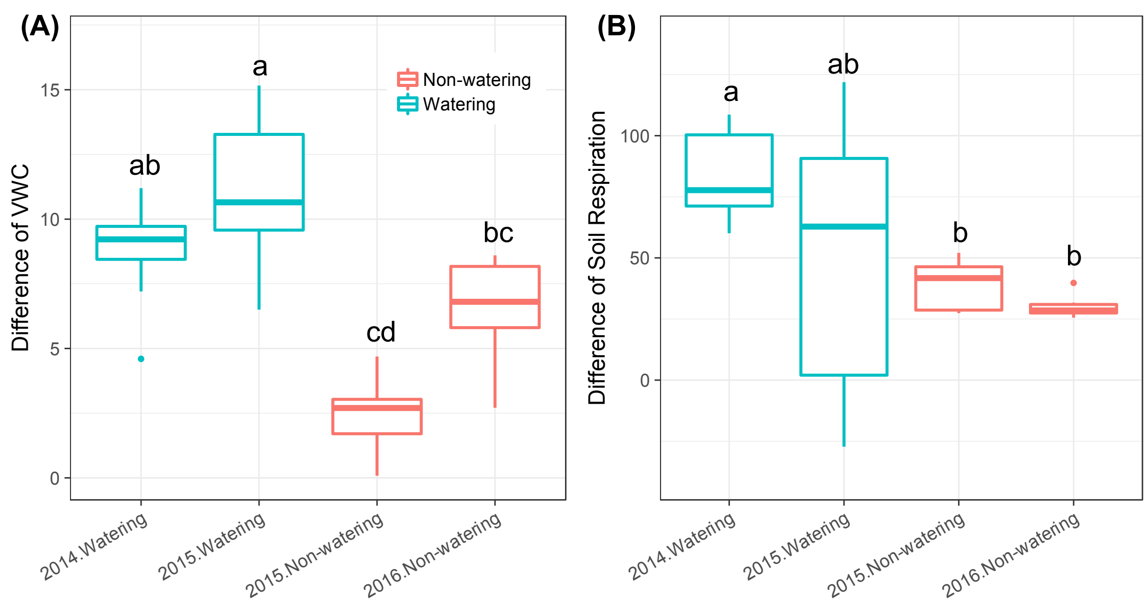


Figure 2-6 Differences of VWC (A) and soil respiration (B) in different years and watering seasons. Different letters represent significant difference at α = 0.05 level

Soil VWC was consistently higher in IP than AP plot in years 2014 and 2015, when extra water was added to IP plot (Figure 2-4). The difference was bigger in 2015 than 2014 (Figure 2-6A) due to more water added in 2015. The difference became smaller when watering stopped in 2015. However, VWC was also consistently higher in IP than AP plot in 2016 non-growing season, when no extra water was added to the plot (Figure 2-4). The reason may be that soil drains differently in the two plots owing to the fact that the IP plot has a several-meter lower elevation and is closer to a ditch (Figure 2-1). It is to be expected the difference of VWC would become smaller in growing seasons in 2016 when transpiration became more intense. Despite the fact that there may exist a difference of VWC initially, adding water did increase the VWC difference in 2015 and 2014 than 2016, though difference was not significantly higher in 2014 than 2016.

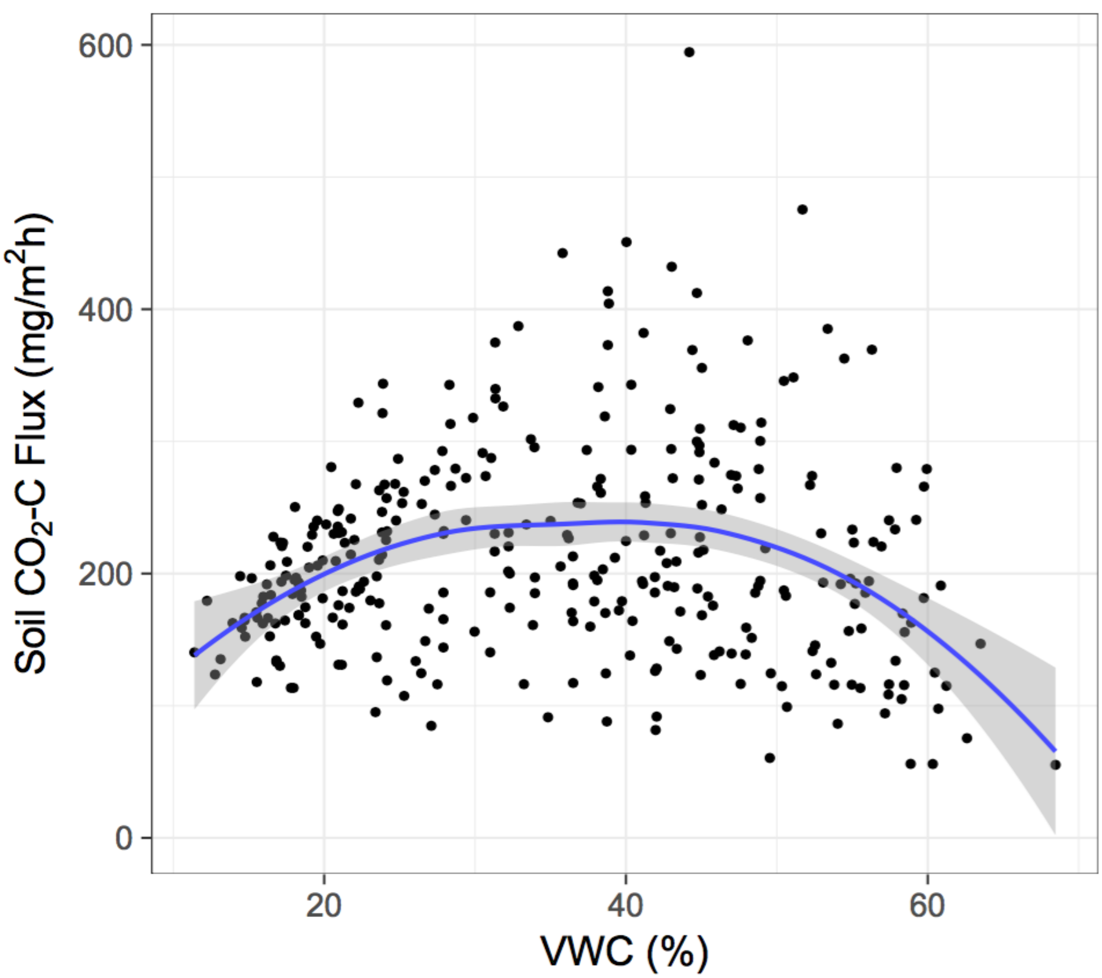


Figure 2-7 Relationship between soil respiration and VWC (R^2 = 0.12).

Generally, decreased precipitation reduces soil respiration, while increased precipitation enhances soil respiration (Vicca et al., 2014a; Z. Wu, Dijkstra, Koch, Penuelas, et al., 2011). Soil respiration was consistently higher in IP than AP plot in all years, even when VWC was not different in 2015 non-watering season (Figure 2-5, Figure 2-6B). A higher root biomass due to a higher soil moisture (Deng et al., 2012) in growing season in IP plot could lead to a higher activity by not only root itself but also microbes with extra carbon sources from roots (Makiranta et al., 2008) in non-watering season, leading to a higher soil respiration even when VWC was not different.

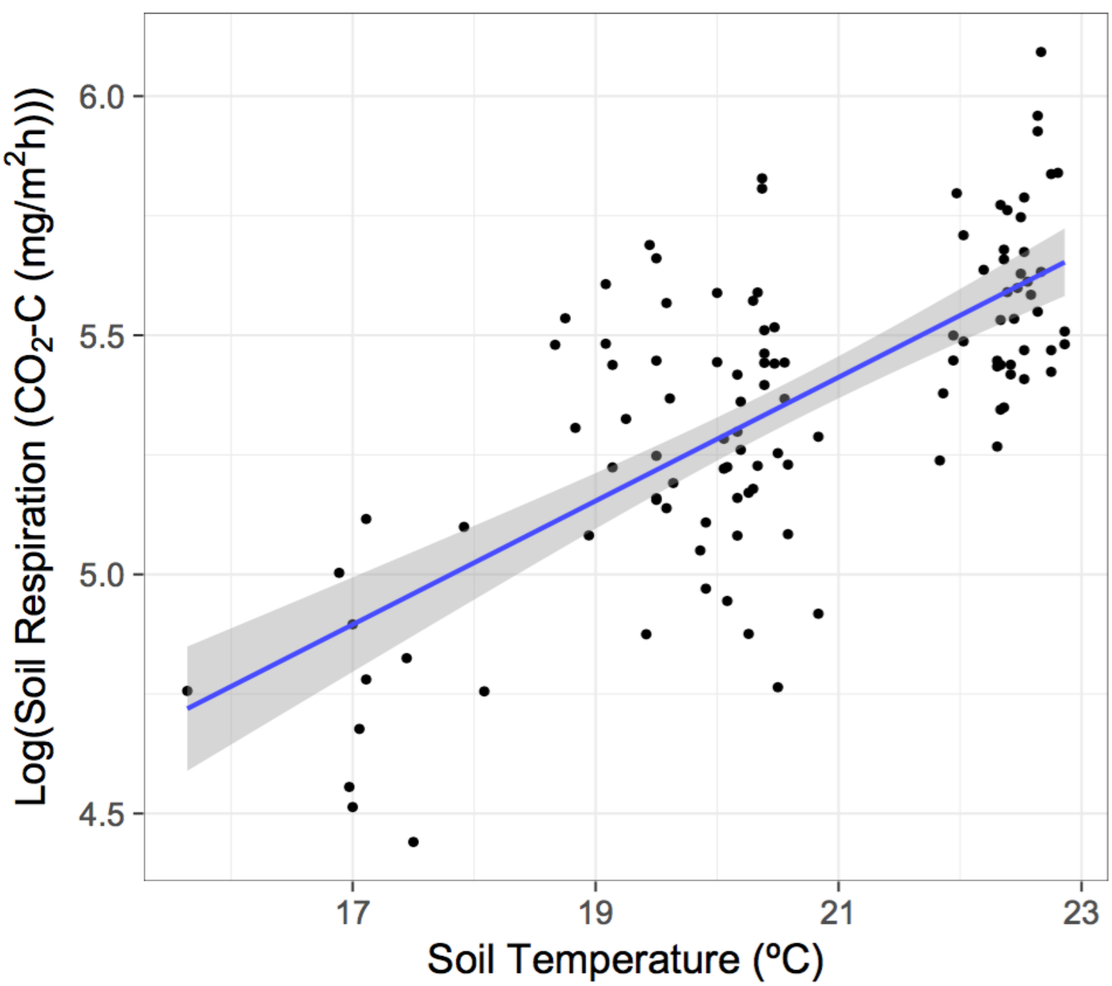


Figure 2-8 Relationship between soil respiration and soil temperature (R^2 = 0.49).

At low moisture, osmotic stress and substrate diffusion limit microbial activity (Moyano et al., 2013; Schimel et al., 2007). Due to reduced root growth and ion uptake, root respiration is also low when soil moisture is low (Thorne & Frank, 2009). At high soil moisture levels, diffusion of oxygen is reduced and thus suppresses microbial (Moyano et al., 2013) and root activity (Makiranta et al., 2008), resulting in low soil respiration. As expected, we found a non-linear response of soil respiration to soil VWC (Figure 2-7), with soil respiration being the highest at around 37% at our site. The quadratic relationship between soil respiration and soil VWC can also explain why the soil respiration difference was the highest in 2014, even though VWC difference was the largest in 2015 watering season (Figure 2-6). Even though more water was added to IP plot in 2015, the increase of cumulative CO_2 (IP – AP) was more in 2014, when soil moisture was not above the optimum most of the time. The difference in soil respiration between two sites was driven not only by difference of VWC, but also by initial VWC at AP plot (Figure 2-9, Table 2-3). At low VWC, when soil moisture is limiting, a higher difference of VWC would lead to a bigger soil respiration difference, which is the case in 2014. However, when soil moisture is above the optimum, the increase of VWC would cause no difference or even a decrease of soil respiration. An example of this happened in June and early July in 2015. June in 2015, with the precipitation of 164.1 mm, was the wettest month in the two years 2014 and 2015, leading to a high VWC value above threshold in AP and IP plots in June and early July. Even though the difference of VWC was high during that time, lack of oxygen for microbes and roots caused the IP plot to have a lower soil respiration.

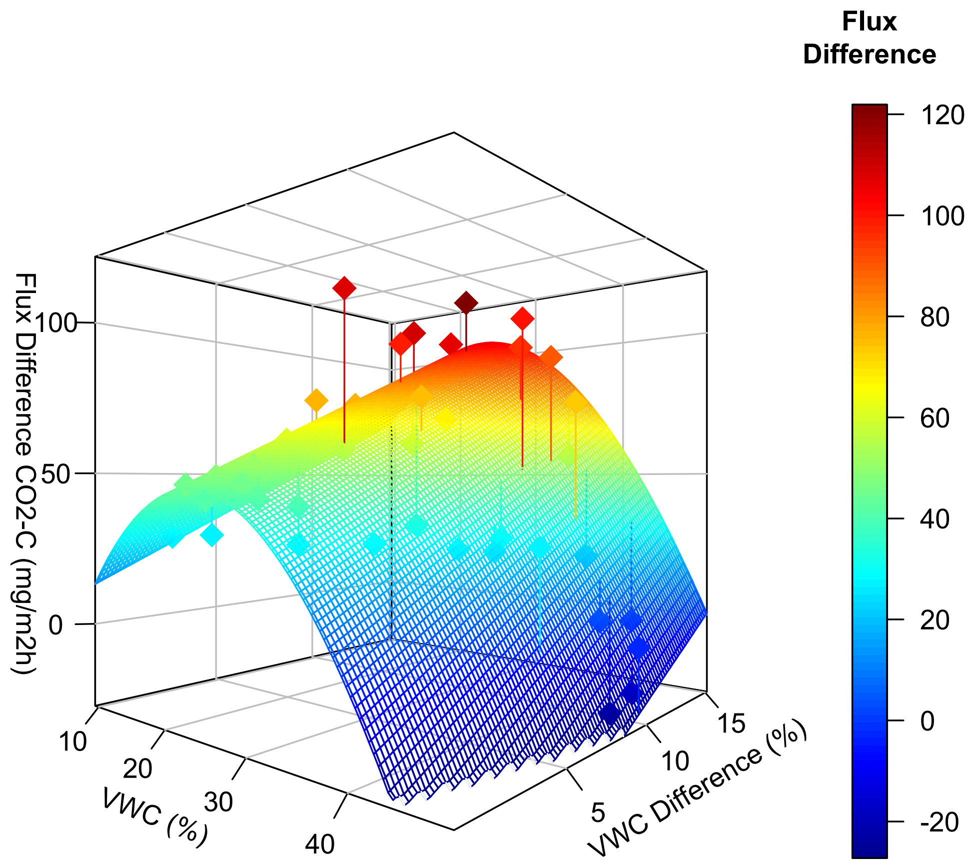


Figure 2-9 Response surface curve formed fitting difference of soil respiration using difference of VWC and VWC at ambient plots (R^2 = 0.63)

Studies to date have highlighted the potential of increased precipitation in arid ecosystems and decrease of precipitation in humid areas to affect carbon balance (L. L. Liu et al., 2016; Vicca et al., 2014a). Our study site is a mesic system with intermediate water levels where soil respiration also strongly responded to the increase of rainfall amount. Soil respiration in mesic systems such as ours could have non-linear response to soil moisture with distinct thresholds (Alan K. Knapp et al., 2008; Suseela, Conant, Wallenstein, & Dukes, 2012), which could be implemented in models to improve predictions of the consequences of projected climatic scenarios for soil respiration in mesic systems.

2.5 Conclusions

Our experiments provided evidence that increased precipitation would affect soil respiration in temperate forest, where soil moisture is not limiting. Soil respiration increased as soil moisture increased to threshold, after which soil respiration decreased due to low availability of oxygen. Increased rainfall increased soil moisture and enhanced soil respiration except when soil moisture was above the threshold. In addition to change of rainfall amount, the frequency and magnitude of extreme rainfall events are expected to increase as temperature continue to increase (IPCC, 2013). Future experiments should be carried out to manipulate frequency and intensity of rainfall events on soil carbon cycling processes, including soil respiration.

Acknowledgements

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3. The partitioning of litter carbon fates during decomposition under different rainfall patterns: a laboratory study

Abstract

During litter decomposition, three major fates of litter carbon (C) are possible: emission as carbon dioxide (CO_2) into the atmosphere, leaching of dissolved organic carbon (DOC), and translocation and transformation into soil organic carbon (SOC). Soil moisture, one of the key drivers of litter decomposition, is predicted to change in the future due to shifts in precipitation patterns. The effects of altered rainfall patterns, especially the increase of extreme events on partitioning of different fates of litter C have never been explored. Here we report the partitioning of litter carbon fates under three different rainfall patterns using soils collected from a temperate forest in the northeastern United States with ^{13}C-labeled tulip poplar litter. More litter derived carbon was recovered in deeper soil and in the DOC pool under extreme rainfall. The leaching of labile carbon caused priming, and the effect was weaker in extreme treatment. This combined with possible more intense physical transfer of particulate organic matter resulted in higher total C content at surface soil in extreme rainfall. Our results highlight that extreme precipitation events affect partitioning of litter carbon. Increase of extreme rainfall events, as projected by most climate models, may lead to altered carbon cycling in temperate forest soils.

3.1 Introduction

Leaf litter decomposition is a key component in the cycling of terrestrial carbon and other nutrients. During decomposition, leaf litter is first fragmented by organisms or abiotic factors such as wind, light, and rain (Cortez, 1998; Cortez & Bouche, 1998; Schadler & Brandl,

2005) on the soil surface and then decomposed and transformed to complex organics and atmospheric CO_2 by bacteria and fungi (Rubino et al., 2010). Labile litter carbon leached to the soil can be incorporated into microbial biomass, or stabilized by interactions with clay minerals (M. Francesca Cotrufo et al., 2015; M. F. Cotrufo et al., 2013). When the litter carbon is incorporated into microbes, it is partially mineralized to provide energy for the microbes, releasing CO_2 into the atmosphere. Overall, during litter decomposition, three major pathways contribute to leaf carbon loss: (1) DOC leached out of soil (2) carbon in organic compounds translocated into soil as DOC to ultimately form soil organic matter (SOM) and (3) CO_2 released to atmosphere. Partitioning litter carbon among different carbon fluxes is therefore necessary to understand carbon cycling and the contribution of the soil ecosystem to atmospheric CO_2.

Litter decomposition rate is controlled by a multitude of factors including substrate quality (Hu et al., 2018; Prescott, 2010), diversity and activity of soil biota (Ayres, Dromph, & Bardgett, 2006), and climatic variables such as temperature, moisture and actual evapotranspiration (Bjorn Berg, 2000; Cortez, 1998). Soil moisture is driven by the amount, timing, variability and extremity of precipitation. Increased frequency and intensity of intense rainfall events, has already been observed to be widely occurring even in places where total precipitation was decreasing (Trenberth, 2011). Future water regimes projected by Global Circulation Models (GCM) are characterized by an increase in frequency of intense rainfall events both globally (Beier et al., 2012; Huntington, 2006) and regionally in the Northeastern United States (Hayhoe et al., 2006).

Most studies manipulating precipitation regimes have focused on changing total amounts of precipitation through water addition or removal (Beier et al., 2012; Z. Wu, Dijkstra, Koch, PeÑUelas, et al., 2011) while rainfall frequency and intensity has been less studied. Lensing and Wise (2007) found that litter under ambient condition decayed faster than either low-rainfall or high-rainfall treatments perhaps because precipitation at ambient condition was more variable in timing and intensity. In a prairie ecosystem, increased rainfall variability resulted in litter with a higher C:N ratio and a lower decay rate, suggesting temporal distribution of rainfall could alter carbon cycling through its combined effects on litter quality and environmental conditions (Schuster, 2016). However, the effect of increasing frequency and intensity of extreme rainfall events on partitioning of litter carbon during decomposition is not well understood.

Extreme precipitation may trigger changes in microbial activity, physical and chemical soil properties, which can affect mineralization of soil organic matter and leaching losses during wetting period. After a single intense storm, CO_2 efflux from mineralization of litter carbon and soil organic matter could amount to 5-10% of the annual net ecosystem production of mid-latitude forests (Lee, Wu, Sigler, Oishi, & Siccama, 2004). Hentschel, Borken, and Matzner (2007) found that mean dissolved organic carbon concentrations increased in the O horizon as irrigation rate increased and suggested additional DOC input from organic horizons to the mineral soil owing to intense precipitation.

Adding labile litter C during the initial phase of litter decomposition may stimulate the mineralization of soil organic matter, causing 'priming effect', in which addition of an easily decomposable energy source results in increased mineralization rate (Yakov Kuzyakov, 2010; Y. Kuzyakov, Friedel, & Stahr, 2000). Crow et al. (2009) and Sulzman, Brant, Bowden, and Lajtha (2005) found aboveground C input from leaf litter to be an efficient C source inducing priming effect due to increased microbial biomass or activity. Different rainfall patterns could affect the fates of easily decomposable litter carbon, thus altering the magnitude of priming effect, and eventually soil carbon stocks.

Although it is generally expected that different rainfall patterns lead to altered soil carbon fluxes (Harper, Blair, Fay, Knapp, & Carlisle, 2005; Knapp et al., 2002), quantifying litter carbon fates into CO_2, soil organic matter, and DOC is challenging. The application of ^{13}C isotopes provides a useful tool for exploring the fates of litter carbon during decomposition. For example, Bird, Kleber, and Torn (2008) used ^{13}C labeled root and needle to accurately quantify labeled C in different soil organic matter fractions in a forest soil; with ^{13}C labeled leaf litter, studies yielded a better estimate of litter carbon partitioning and found mineralization of litter carbon to be the major pathway of carbon loss (Adrian Kammer, Schmidt, & Hagedorn, 2011; Rubino et al., 2010).

We performed a lab experiment to partition litter carbon fates in different manipulated rainfall patterns. To achieve this, we incubated ^{13}C-labeled leaf litter in direct contact with the soil and thereafter partitioned the fate of litter-carbon into SOC, CO_2 and leachate-DOC based on ^{13}C mass balance. Decades of historical precipitation data was used to determine control, medium and extreme treatments. The total amount was kept constant, while control treatment was composed of low intensity, high frequency rainfall events, extreme treatment was composed of high intensity, low frequency rainfall events, and medium treatment was alternating between the two. We hypothesized that under extreme precipitation (1) litter would decompose more slowly (2) more carbon would be lost as DOC (3) more litter carbon would be transported to deeper layers, and (4) less priming effect would happen due to (1) and

(2). To our knowledge, this is the first litter decomposition study in which mass loss has been budgeted in terms of CO_2 efflux to the atmosphere, C input to the soil to form soil organic matter, and DOC leached out of soil system under different rainfall patterns. This represents an important step towards a deeper understanding of soil carbon cycling under change of rainfall patterns, especially the increase of extreme events.

3.2 Materials and methods

3.2.1 Research site and soils

Soils from a mature forest stand at the Smithsonian Environmental Research Center (SERC) were collected for this laboratory study. SERC is located along the western shore of Chesapeake Bay in Edgewater, MD (38º53'N, 76º33'W) with a mean annual precipitation of 1,146 mm and the mean annual temperature of 13ºC (Correll et al. unpublished data). The site we collected our soils is a 150-year forest stand dominated by tulip poplar (*Liriodendron tulipifera*), sweet gum (*Liquidambar styraciflua*), oaks (*Quercus spp*.), American beech (*Fagus grandifolia*), and hickories (*Carya spp*.) (Pitz et al., 2018). The soil type is Collington (Typic Hapludult, fine sandy loam) (Yesilonis et al., 2016). Soil properties are described in Yesilonis et al. (2016).

3.2.2 Laboratory experiment setup

To detect measurable changes in soil carbon content we set up six months long experiment in medium size mesocosms, which are often used in soil ecology and biogeochemistry experiments (Crumsey et al., 2015; Setälä, Martikainen, Tyynismaa, & Huhta, 1990). Taking undisturbed soil monoliths of this size from forests is challenging due to the high density of roots. As a compromise between undisturbed and completely homogenized soils, we recreated soil horizons with homogenized soils following the so-called 'simulated forest floor' approach proposed by Huhta and Setälä (1990). Specifically, soils were collected from our study site in September 2016 from the top 10 cm (surface soil) and from the deeper (20-40 cm) mineral layer (subsurface soil). Both soil layers were sieved through a 4 mm sieve with roots and leaves removed. Soil was reconstructed in transparent acrylic columns, with a diameter of 19.0 cm (Figure 3-1). At the bottom of the acrylic column, a 10 cm high clean gravel layer was added to filter leachate and prevent anaerobic conditions. The gravel was covered with 2 mm mesh and topped with 6.0 and 2.8 kg subsurface and surface soil, respectively. This amounted to a 25 cm high soil column (15 and 10 cm), with bulk densities similar to those measured in the field at SERC's mature forests of 1.32 and 0.78 g cm^{-3} at 10-25 cm and 0-10 cm depth respectively. Carbon contents were 4.1% and 0.5%, and $\delta^{13}C$ are -27.3‰ and -26.0‰ at 0-10 cm and 10-25 cm depth, respectively.

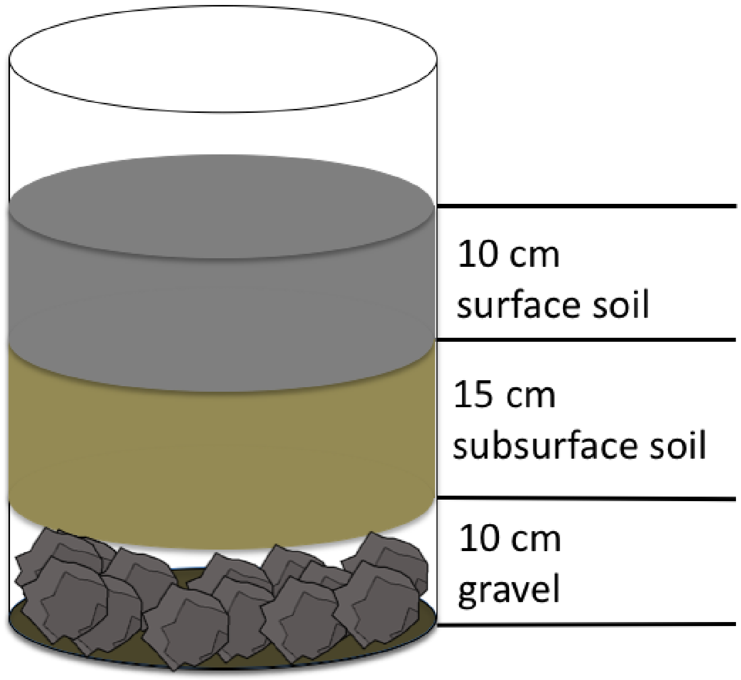

Figure 3-1 Set up of soil column

Lab temperature and relative humidity were monitored continuously by Maxim's iButton (Maxim Integrated, San Jose, California, USA). ECH_2O EC-5 moisture sensors (METER Group, Pullman, Washington, USA) were installed at 8 cm depth to monitor volumetric water content (VWC). Leachates were collected using plastic bottles after rainfall events. Thirty milliliters subsamples were immediately passed through 0.45µm glass fiber filters, acidified with phosphate, and stored at 4 ºC for later isotope and concentration analyses.

3.2.3 Historical precipitation data in SERC to determine control and extreme rainfall treatments

The three rainfall treatments established in the lab were based on historical precipitation data from SERC and surrounding weather stations. Fifteen-minute precipitation data from US Custom House in Baltimore (1984 – 1999) (https://www.ncdc.noaa.gov/cdo-web/datatools) was used to determine rainfall intensity. Frequency of heavy rainfall events was derived from daily precipitation data from US Naval Academy (1894 – 1976) (https://www.ncdc.noaa.gov/cdo-web/datatools) in Annapolis, Maryland and SERC (2002 – 2013). Table 3-1 demonstrates how control and extreme rainfall treatments are comparable to historical precipitation data. Both the frequency and intensity of rainfall were manipulated, while total amount remained constant. Control treatment had an average of 15-minute rainfall intensity and average frequency of rainy days. The extreme treatment had an intensity and frequency of top 1% of 15-minute rainfall intensity and top 1% of frequency of rainy days respectively. The medium rainfall treatment was alternating between control and extreme rainfall treatments in a two-week interval. As a result, during a four-week period, the extreme treatments received two heavy rainfall events with high intensity, the control treatments received eight rainfall events with low intensity, and the medium treatments received four rainfall events with low intensity in the first two weeks, followed by one heavy rainfall event with high intensity (Figure 3-2).

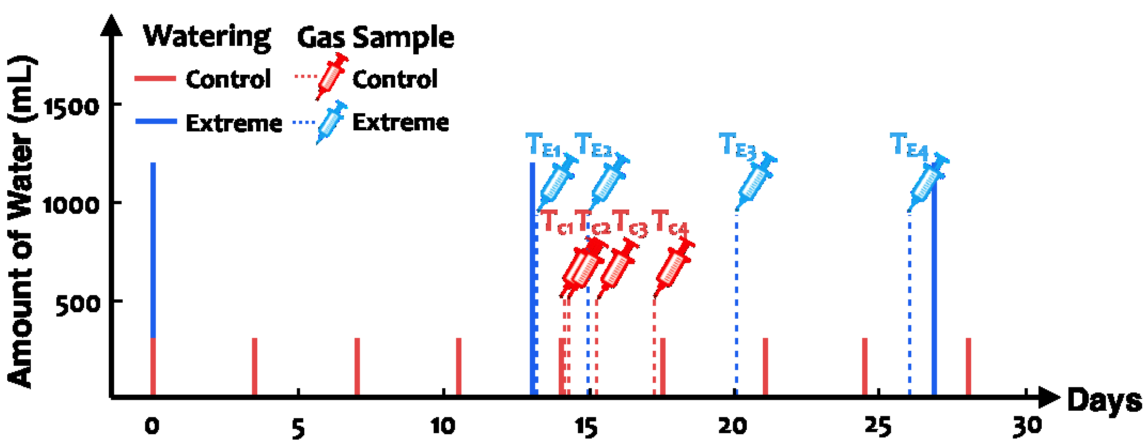

Figure 3-2 Timeline of first 30 days. Medium treatment was alternating between control and extreme treatment in two-week intervals and is not shown in this figure. Throughout the experiment, to avoid complete saturation and anoxic conditions, we stopped watering for one week or reduced the amount to one third. Reduced watering happened on weeks 3, 4, 5, 6, 9,

10, 11, 12, 14, 15, 17, 18, 20, 21, 23, 24 during the six-month long experiment and no watering happened on weeks 13, 16, 19, and 22.

Table 3-1 Historical precipitation data were used to determine rainfall intensity treatments

Rainfall treatments		Manipulations	Historical precipitation data
Intensity (mm/hour)	Control	4.0	3.5 (mean)
	Extreme	30.0	27.4 (top 1%)
Frequency	Control	Once every 3.5 days	Once every 3.3 days (mean)
	Extreme	Once every 14.0 days	Once every 16.0 days (top 1%)

Data source for rainfall intensity: 15-minute precipitation data from US Custom House in Baltimore (1984 – 1999) (https://www.ncdc.noaa.gov/cdo-web/datatools). Data source for frequency of heavy rainfall events: daily precipitation data from US Naval Academy (1894 – 1976) (https://www.ncdc.noaa.gov/cdo-web/datatools) in Annapolis, Maryland and SERC (2002 – 2013)

3.2.4 Rainfall manipulation

Soil moisture was first raised to field capacity by gradually adding distilled water over the course of 20 days. Upon reaching field capacity, 7.0 g (dry mass) of tulip poplar *(L. tulipifera)* leaf litter was placed on the soil surface in litter treatment columns, equaling to a density of 233 g m^{-2}, similar to the tulip poplar litter fall input in old forest stands at SERC (Szlavecz et al., 2018). To follow the fate of litter carbon, the leaf litter was ^{13}C-labeled as described in Bernard, Pitz, Chang, and Szlavecz (2015). Leaves were broken up by hand and sieved through an 8-mm sieve with petioles removed, to ensure relatively homogeneous litter quality. $\delta^{13}C$ and carbon content of leaf litter are 244.2‰ enriched and 43.2%.

There were three rainfall manipulations, control, medium, and extreme, and two leaf litter manipulations, with three replicates in each combination. The six treatments hereafter are labeled as CL (control-litter), CNL (control-no litter), ML (medium-litter), MNL (medium-no litter), EL (extreme-litter), and ENL (extreme-no litter). In the extreme rainfall event, 1200 ml deionized water was added to column in 1.5 hours. In the control rainfall event, 300 ml water was added to column in 2.5 hours. In both treatments, water was added by a 50 ml syringe gradually in 15-minute intervals to be evenly distributed. When soil moisture reached 35%, we stopped watering for one week or reduced the amount to one third to avoid complete saturation and anoxic conditions (Figure 3-2). Rainfall manipulations started in March and ended in September 2017.

3.2.5 CO_2 flux measurements

CO_2 flux was measured daily, and more frequently before and after rainfall events, with a total of 3663 measurements made. Static chamber method was used to determine CO_2 fluxes. A PVC lid assembled with a CO_2 sensor (GMP 343, Vaisala, Vantaa, Finland) was placed on the experimental columns. CO_2 concentrations in the headspace were recorded every second for 6 minutes. Then CO_2 concentration was averaged in 30 seconds intervals to account for the fluctuation of readings from sensors, especially when flux was low. Gas flux rate was calculated as:

$$F = \frac{dC}{dT} * \frac{V}{S}$$

where *F* is the gas flux in mg m^{-2} h^{-1}, *C* is the mole concentration in µmol m^{-3}, *T* is time, *V* is the volume of headspace, and *S* is the area of the soil surface in the chamber. *dC/dT* can be estimated as slopes of fitted lines between CO_2 concentrations and time.

3.2.6 $\delta^{13}C$ of respired CO_2 measurements

$\delta^{13}C$ of respired CO_2 was measured approximate monthly from March to August 2017 for a total of five campaigns. When taking $\delta^{13}C$ measurement, an airtight lid with septa was placed on top of the plastic column. A total of three or four gas samples of 60 ml were collected at different CO_2 concentrations using Cali-5-Bond air & gas sampling bags (Calibrated Instruments Inc., McHenry, Maryland, USA). CO_2 concentration and $\delta^{13}C$ of CO_2 was determined by a cavity ring-down spectroscopic carbon isotope analyzer (Picarro G2101-i, Picarro Inc., Santa Clara, California, USA) connected to an automated sampling manifold (Picarro A0311). We used keeling plots to calculate $\delta^{13}C$ of respired CO_2 (Brand & Coplen, 2012). We occasionally dropped a data point because of poor quality data from the analyzer. In every case, the slope was based on ≥ 3 observations. For a slope to be determined as a quality data point, the R^2 had to be greater than 0.80.

To gain insight on how $\delta^{13}C$ of respired CO_2 and proportion of CO_2 from leaf litter would change after rainfall events, four time points were selected for gas sampling in both control and extreme rainfall in litter addition treatments. In general, $\delta^{13}C$ of respired CO_2 was measured right after (T_{C1}), 4 hours after (T_{C2}), 1 day after (T_{C3}), and 3.5 days after (T_{C4}) control columns received a rainfall treatment (Figure 3-2). While for extreme rainfall treatment, $\delta^{13}C$ of respired CO_2 was measured right after (T_{E1}), 2 days after (T_{E2}), 1 week after (T_{E3}), and 2 weeks after (T_{E4}) rainfall event (Figure 3-2). In weeks 3-4, $\delta^{13}C$ of respired CO_2 was only measured in one column in each treatment. In weeks 7-8, two columns were measured in each treatment. For the following three campaigns of measurements in weeks 11-12, 17-18, and 23-24, all three columns in each treatment were measured.

3.2.7 Litter collection and soil sampling

In September 2017 at the conclusion of the experiment, all recognizable litter residues on the soil surface within each column were collected. All the soil was divided into 5 depths: 0-2, 2-6, 6-12, 12-18, and 18-25 cm. The collected litter residues and subsamples of soil samples were oven dried at 70 ºC to constant weight and ground for later isotope analyses. Gravimetric water content (GWC) was determined by drying a subsample of soil samples at all depths at 105 ºC until constant mass for recovery of dry soil mass and soil carbon.

3.2.8 Stable isotope analyses of soils, leaves, and DOC

The C stable isotope compositions of soils, leaves, and DOC from the mesocosm experiment were analyzed at the UC Davis Stable Isotope Facility (Davis, California, USA). Leaves were analyzed for ^{13}C isotope using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Soils were analyzed for ^{13}C isotope using an Elementar Vario EL Cube or Micro Cube elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer. DOC was analyzed for ^{13}C using an O.I. Analytical Model 1030 TOC Analyzer (Xylem Analytics, College Station, TX) interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer utilizing a GD-100 Gas Trap Interface (Garden Instruments). Samples were acidified and purged with helium off-line to remove all dissolved inorganic carbon. Stable isotope ratio of C was expressed using delta (δ) notation: $\delta^{13}C_{sam} = [R_{sam}/R_{std} – 1] * 1000‰$, where R_{sam} is the isotope ratio ($^{13}C/^{12}C$) in the samples, and R_{std} is the isotope ratio in the standard, which is Pee Dee Belemnite (PDB) for C.

3.2.9 Calculations

SOC-C

The fraction of litter-derived C in the different soil layers f_s can be obtained by applying a two source mixing model (Balesdent, Mariotti, & Guillet, 1987) of the difference in δ values between the soil with litter (δ_s) and the average of no litter treatment soil (δ_n) at the end of the experiment, according to:

$$f_s = \frac{\delta_s - \delta_n}{\delta_l - \delta_n}$$

where δ_l is the $\delta^{13}C$ value of the litter sample. We assumed the $\delta^{13}C$ values of the litter-derived C incorporated into SOM is equivalent to the $\delta^{13}C$ values of the bulk litter. The amount of carbon from leaf litter at different layers $M_{l\text{-}s}$ was calculated as:

$$M_{l-s} = M_{s-s} \times (1 - GWC) \times C_{carbon-s} \times f_s$$

where $M_{s\text{-}s}$ is the mass of soil, $C_{carbon\text{-}s}$ is carbon content (g/g) of soil.

Leachate-C

The fraction of leachate derived from litter f_l can be estimated by:

$$f_l = \frac{\delta_{s-l} - \delta_{n-l}}{\delta_l - \delta_{n-l}}$$

where $\delta_{s\text{-}l}$ is $\delta^{13}C$ of DOC from litter treatment soils, $\delta_{n\text{-}l}$ is $\delta^{13}C$ of DOC from no litter treatment soils, and δ_l is the $\delta^{13}C$ value of the litter sample. We assumed that the $\delta^{13}C$ values of the litter-derived leachate are equivalent to the $\delta^{13}C$ values of the bulk litter. The amount of carbon from leaf litter in DOC was calculated as the sum of litter carbon in leachate $M_{l\text{-}l}$:

$$M_{l-l} = V \times C_{carbon-l} \times f_l$$

where V is volume of leachate, and $C_{carbon\text{-}l}$ is the concentration of DOC.

CO_2-C

The amount of carbon from leaf litter in CO_2 (M_{CO2}) was calculated from mass balance:

$$M_{CO2} = M_{carbon-loss} - \sum M_{l-s} - \sum M_{l-l}$$

where $M_{carbon\text{-}loss}$ was calculated as the difference between litter carbon before and after incubation.

Priming effect

The fraction of litter-derived CO_2 over the CO_2 respired f_{CO2} can be estimated by:

$$f_{CO2} = \frac{\delta CO2_s - \delta CO2_n}{\delta CO2_l - \delta CO2_n}$$

where $\delta CO2_s$ is the $\delta^{13}C$ value of respired CO_2 from the soil with leaf litter, $\delta CO2_n$ is the $\delta^{13}C$ value of respired CO_2 from no litter treatment soil, which is estimated as δ value of initial soil at surface, and $\delta CO2_l$ is the $\delta^{13}C$ value of respired CO_2 from labeled litter. We assumed that no isotopic fractionation is associated to the respiration process ($\delta CO2_l = \delta_l$, $\delta CO2_n = \delta_n$).

Priming effect (*PE*) was calculated as the difference between CO_2 efflux from soil with and without litter of the same rainfall treatment at the same time (Yakov Kuzyakov, 2010; Y. Kuzyakov et al., 2000):

$$PE = (1 - f_{CO2}) \times E_l - E_n$$

where E_l and E_n are CO_2 efflux from soil with and without litter addition, respectively.

3.2.10 Statistical analysis

All statistical analyses were conducted using R version 3.3.3 (R Core Team, 2017). P values below 0.05 were considered significant and those between 0.05 and 0.1 were considered nearly significant. One-way ANOVA was used to evaluate effect of rainfall on litter mass loss, cumulative CO_2 efflux, and different components of recovered litter carbon in litter treatment soils. Two-way ANOVA was used to evaluate effects of both depth and litter on $\delta^{13}C$ and carbon content of soil. Effects of litter and rainfall on total volume and carbon mass of leachate, and carbon content at 0-2 cm depth were also evaluated by two-way ANOVA. Mixed effect models were conducted using the *lme4* package (Bates, Machler, Bolker, & Walker, 2015) to assess rainfall effect on priming effect. Priming effect was compared between T_{C1} and T_{E1} (right after water addition), and between T_{C3} and T_{E2} (1 day and 2 days after water addition respectively). Column number was treated as random effect, sampling weeks, sampling time (whether T_{c1} and T_{E1} or T_{c3} and T_{E2}), and rainfall were treated as fixed effects. Factors were evaluated in the models in the above order. Mixed effect models were also run to evaluate effects of sampling weeks, sampling time, and rainfall on proportion of CO_2 from leaf litter, with the same factors evaluation order as before. Linear regression model was run to explore the relationship between DOC concentration and volume of leachate.

3.3 Results

3.3.1 Litter mass loss and recovery of litter carbon

After six months, tulip poplar leaf litter lost 67.3 ± 3.2 % mass in control rainfall treatment, 64.7 ± 3.2 % in medium rainfall treatment, and 60.8 ± 3.4 % in extreme rainfall treatment. Litter mass loss showed no significant difference among three rainfall treatments.

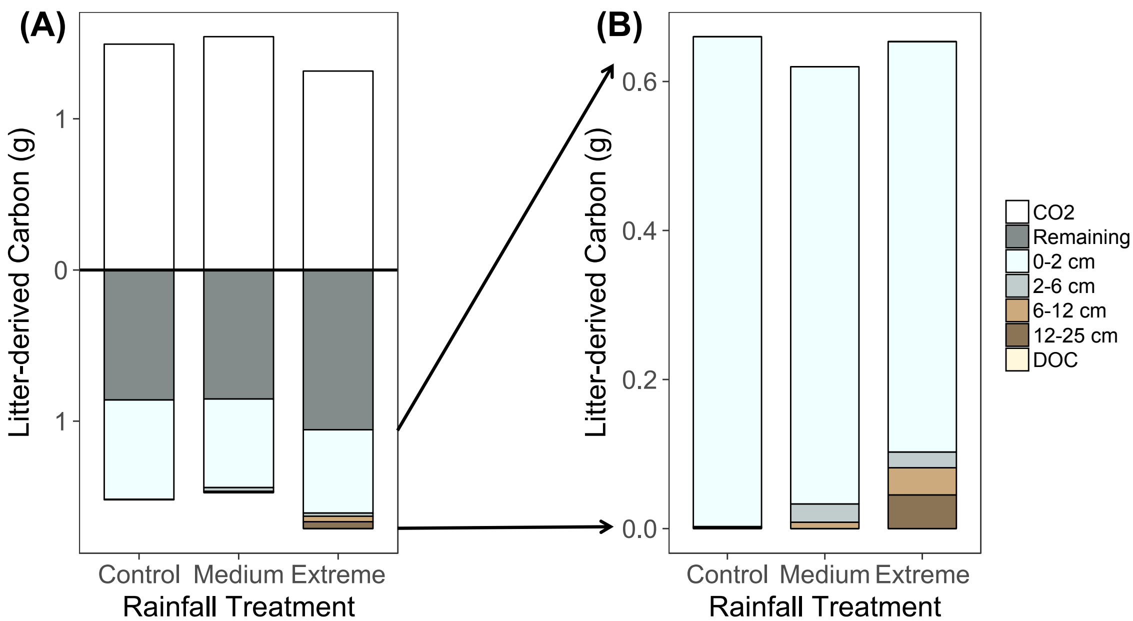

Figure 3-3 Litter-derived carbon in all components (A) and soil (B) in different treatments. In plot A, the bar above zero line represents aboveground while the bar below zero line means soil surface and belowground. Plot B is an enlarged part of plot A, with just soil included. Note the small contribution of litter carbon to DOC.

Based on mass balance, about half of litter carbon (48.1 ± 1.5%) was respired as CO_2, with remaining leaf litter (30.6 ± 1.8%) and soil carbon (21.3 ± 1.2%) mostly comprising the other half (Figure 3-3A). Only a small fraction (0.02 ± 0.01%) of litter carbon ended up in DOC. A comparison between the extreme and control rainfall treatments (Figure 3-3B) reveals that more litter carbon was recovered from the extreme at 2-6 cm, 6-12 cm, 12-25 cm soil depths and in DOC. Details of each pathway of litter carbon are discussed later.

3.3.2 CO_2 efflux

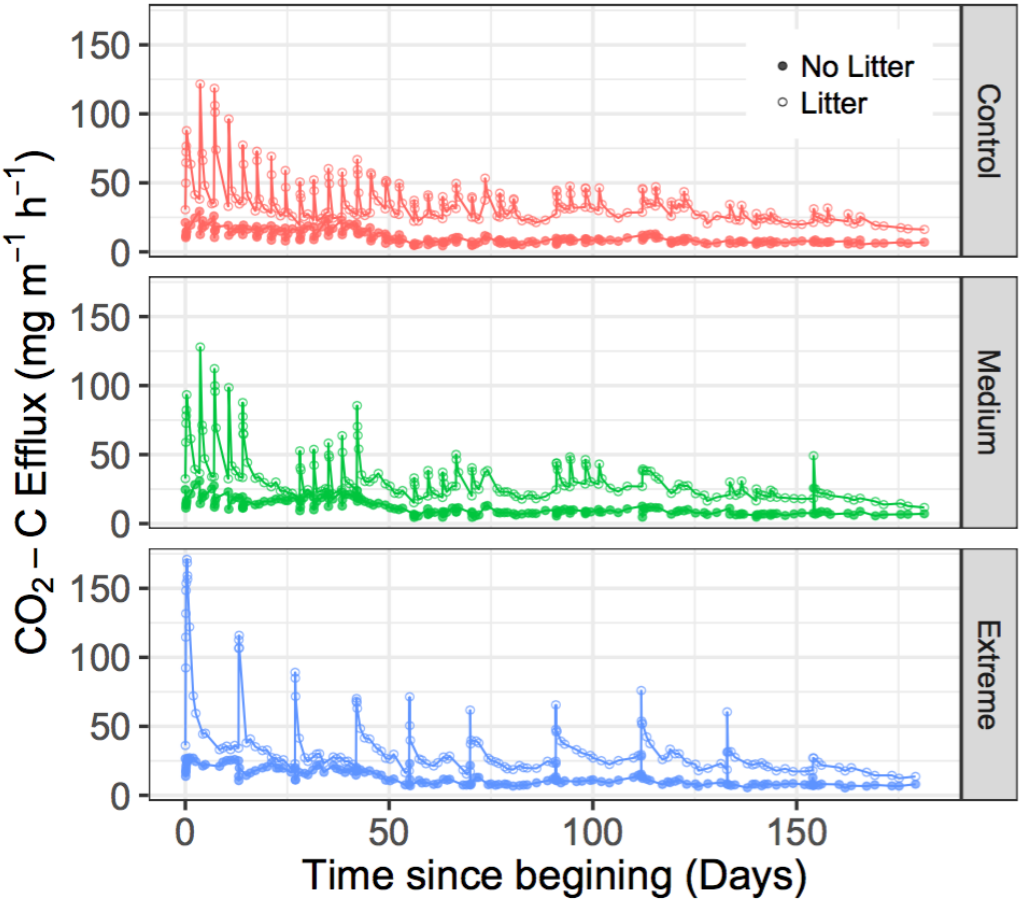

Figure 3-4 CO_2 efflux during lab experiment in different rainfall treatments. Each peak indicates a rainfall application. Each point is the mean of three replicate measurements.

After each rainfall event, mesocosms with litter addition exhibited a pulse of CO_2 (Figure 3-4) followed by a rapid decrease as the leaf litter dried. Both litter and no litter treatments soils showed a decreasing trend of CO_2 efflux as the experiment proceeded (Figure 3-4). The cumulative CO_2 efflux during incubation were 3927.1 ± 189.6 mg, 3489.3 ± 164.7 mg, and 3599.3 ± 176.0 mg C-CO_2 for CL, ML, and EL treatments, respectively; the results were not significantly different. Similar to CO_2 flux, the proportion of CO_2 from leaf litter peaked after each rainfall treatment and decreased until next rainfall event (Figure 3-5, Table 3-2), evidenced by $\delta^{13}C$ of respired CO_2 (Figure 3-6). The contribution of leaf litter to CO_2 flux decreased over time (Figure 3-5, Table 3-2).

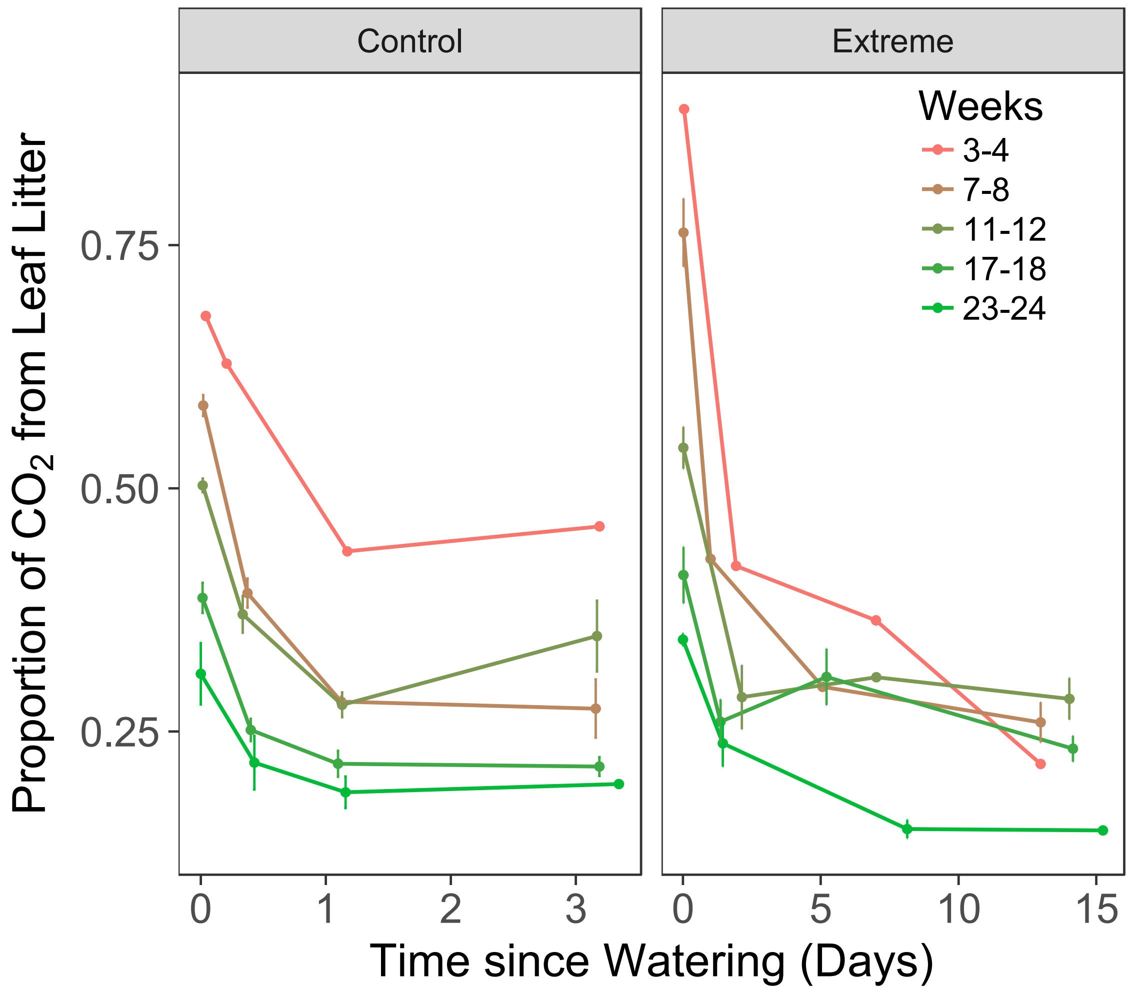

Figure 3-5 Temporal change of proportion of CO_2 from leaf litter after different rainfall treatments. In weeks 3-4, only one column was measured in each treatment; in weeks 7-8, two columns were measured; in the following three campaigns, all three columns were measured. Error bars represent standard error. Note the different scales of the x-axes.

Table 3-2 Results of mixed effect models testing the effects of rainfall on proportion of CO_2 from leaf litter (Prop) and priming effect (PE) (mg m^{-2} h^{-1})

	Weeks		Time		Rainfall	
	χ^2	P	χ^2	P	χ^2	P
Prop	38.65	< 0.001	108.22	< 0.001	0.16	0.685

PE	46.40	< 0.001	3.45	0.063	3.95	0.047

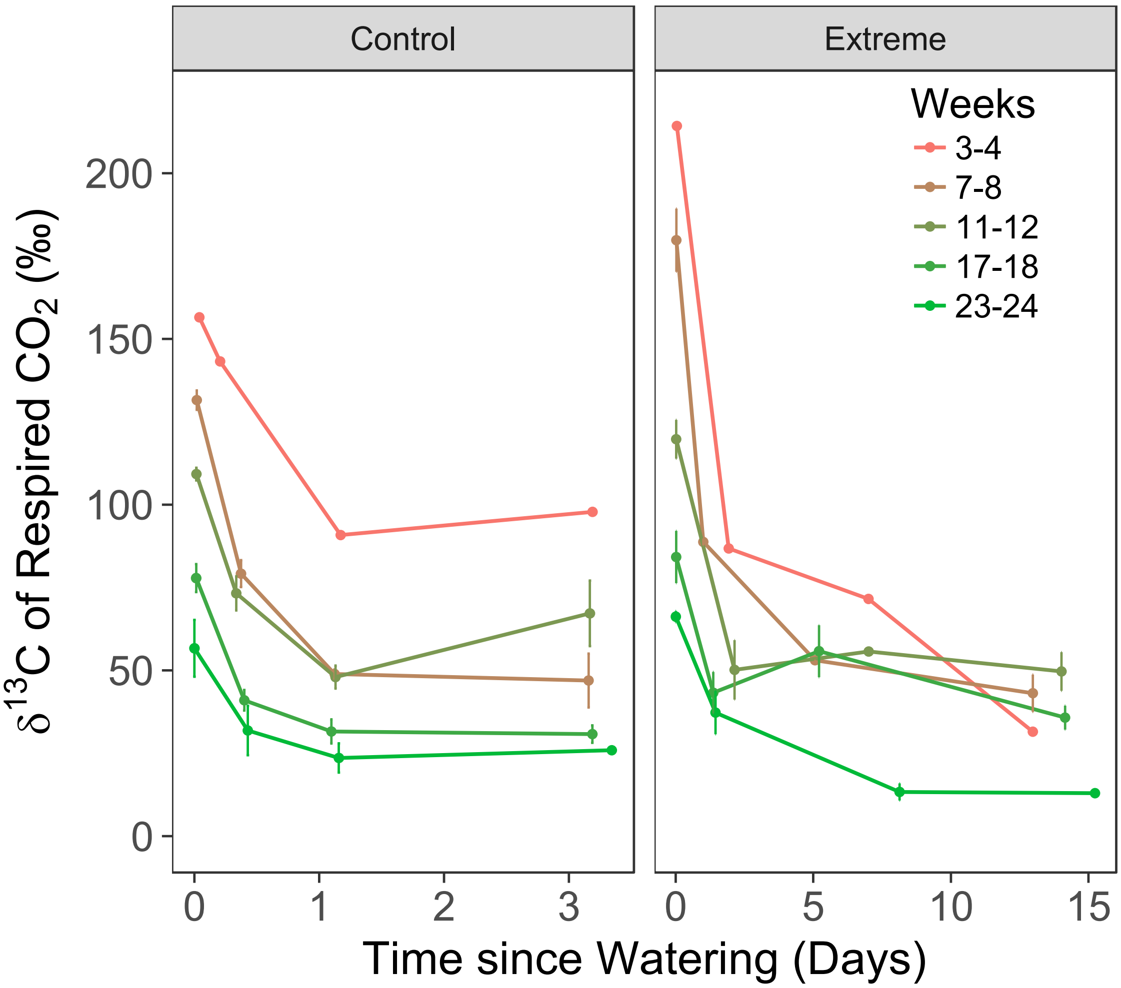

Figure 3-6 Temporal change of $\delta^{13}C$ of CO_2 from litter treatment soils after different rainfall events. In weeks 3-4, only one column was measured in each treatment; in weeks 7-8, two columns were measured; in the following three campaigns, all three columns were measured. Error bars represent standard error. Note the different scales of x-axes.

Priming effect slowly increased and peaked in weeks 17-18 and decreased afterwards, and was higher in the control than in the extreme treatments (Figure 3-7, Table 3-2).

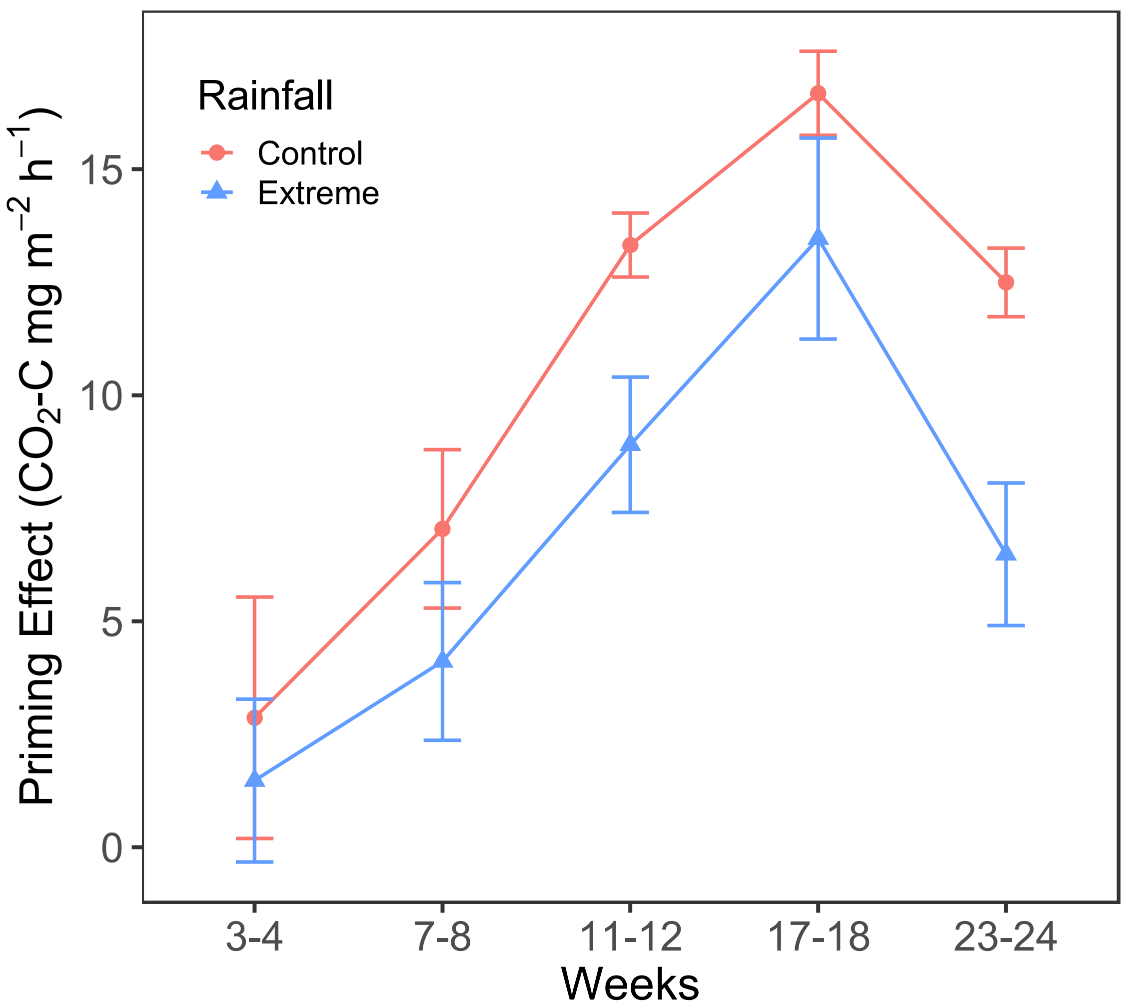

Figure 3-7 Temporal change of priming effect in different rainfall treatments. Priming effect was calculated as the difference of CO_2 efflux from soils with and without litter. The means were calculated from four time points after rainfall application across all columns measured with $\delta^{13}C$ of respired CO_2 (Total N = 96). Error bars represent standard error.

3.3.3 Soil organic carbon (SOC)

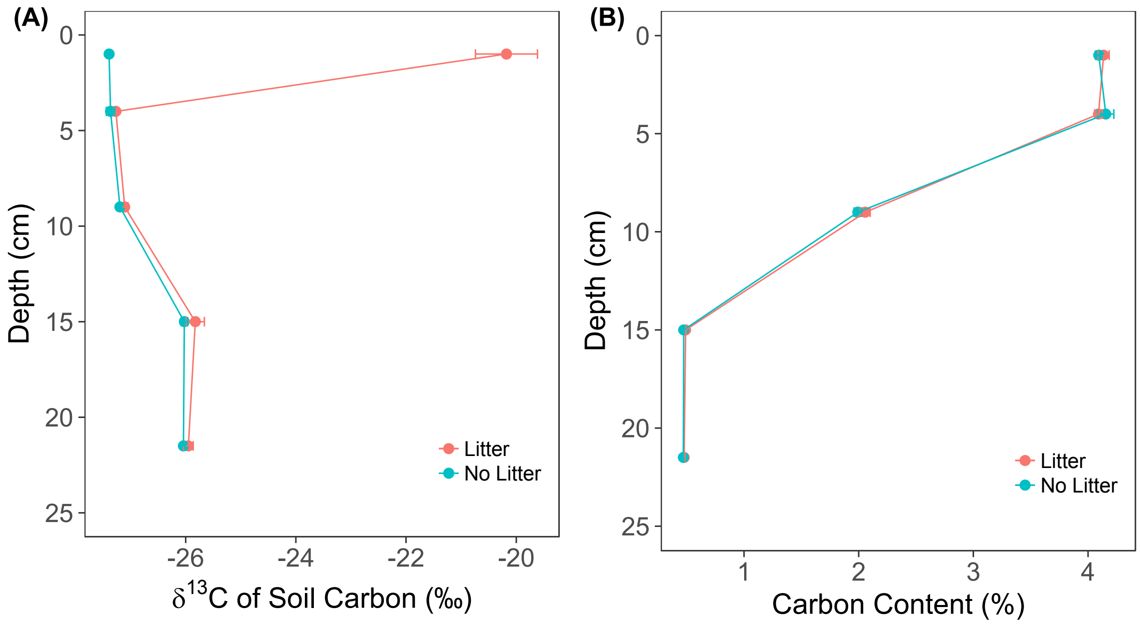

Figure 3-8 $\delta^{13}C$ (A) and carbon content (B) in the soil profile with or without litter addition. Error bars represent standard error (N = 9).

The effects of depth and litter on $\delta^{13}C$ of soil carbon and soil carbon content were evaluated using two-way ANOVA (Figure 3-8). For $\delta^{13}C$, depth ($p < 0.001$), litter ($p < 0.001$), and interaction ($p < 0.001$) effects were all significant. Litter treatment soils had a higher $\delta^{13}C$ value than no litter treatment soils ($p < 0.001$). In no litter treatment soils, $\delta^{13}C$ of soil carbon exhibited an increasing trend from -27.4‰ at surface to -26.0‰ at subsoil. In litter treatment soils, surface soil had the highest $\delta^{13}C$ of -20.2‰; the other depths followed the same pattern as in no litter treatment soils. For soil carbon content, only depth ($p < 0.001$) was significant. On average, soil carbon content decreased from 4.12% at 0-2 cm to 0.47% at 18-25 cm depth.

One-way ANOVA was performed to evaluate the effect of rainfall on $\delta^{13}C$ of soil carbon and soil carbon content in litter treatment soils at different depths separately. For $\delta^{13}C$, rainfall effect was significant at all depths, except at 0-2 cm. In extreme rainfall treatment, $\delta^{13}C$ of soil carbon was higher at 6-12, 12-18, and 18-25 than both control and medium treatment. At 2-6 cm depth, both extreme and medium treatment soils had a higher $\delta^{13}C$ than control treatment soils (Figure 3-9A, Table 3-3). Rainfall effect was only significant on soil carbon content at 0-2 cm. In extreme rainfall treatment, soil carbon content was higher at 0-2 cm than both control ($p = 0.022$) and medium ($p = 0.053$) treatments (Figure 3-9B). Litter addition soils had a higher carbon content ($p = 0.019$) at 0-2 cm than no litter treatment soils only in extreme rainfall treatment.

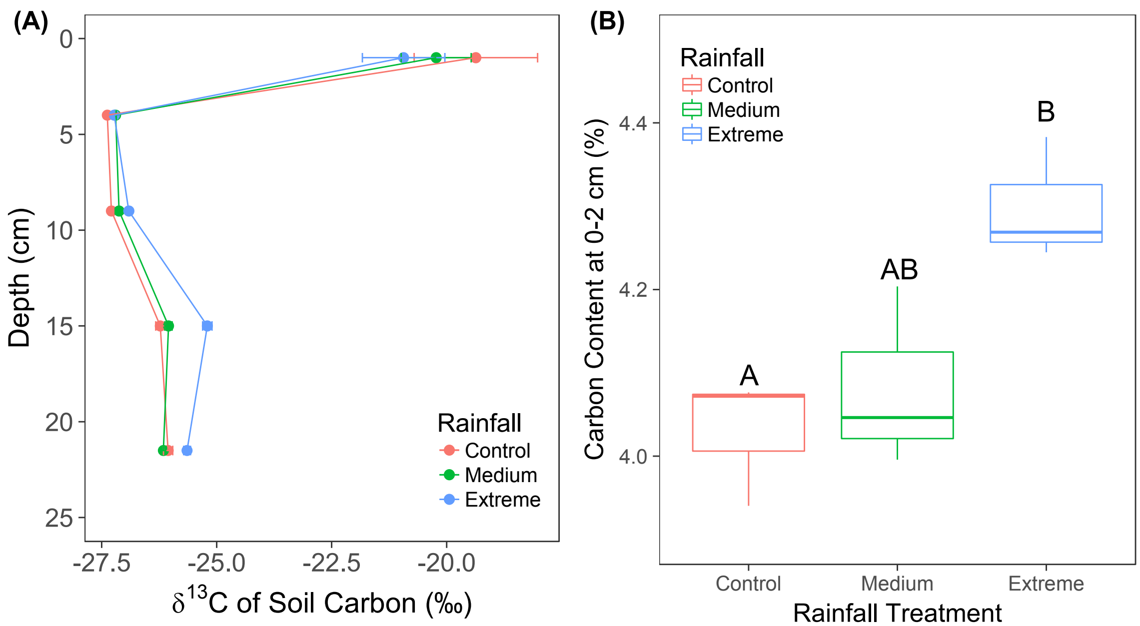

Figure 3-9 $\delta^{13}C$ of soil carbon in the soil profile (A) and carbon content at 0-2 cm (B) in litter addition treatments. Error bars represent standard error. When error bars are not visible they are smaller than the symbol. In plot B, treatments with different letters are significantly different. In plot A, ANOVA tests were all significant except at 0-2 cm, and details of p values are in table 3-3.

Table 3-3 Tukey's test results for comparing $\delta^{13}C$ of soil carbon at different precipitation regimes

Depth (cm) \ Rainfall	Extreme – Control (‰)	Extreme – Medium (‰)	Medium – Control (‰)
0-2	-1.57 (P = 0.561)	-0.71 (P = 0.880)	-0.86 (P = 0.830)
2-6	**0.15 (P < 0.001)**	-0.03 (P = 0.465)	**0.18 (P < 0.001)**
6-12	**0.38 (P = 0.002)**	**0.21 (P = 0.031)**	0.17 (P = 0.079)
12-18	**1.02 (P < 0.001)**	**0.84 (P = 0.002)**	0.18 (P = 0.423)
18-25	**0.41 (P = 0.022)**	**0.51 (P = 0.007)**	-0.10 (P = 0.641)

Significant differences are indicated in bold (P < 0.05)

Similarly, one-way ANOVA was used to test the effect of rainfall on carbon from leaf litter at different depths. At surface soil, no difference existed among different rainfall treatments. At 2-6 cm depth, carbon from leaf litter is higher in both medium and extreme than control treatment soils. At 6-12, 12-18, and 18-25 cm depths, carbon from leaf litter is higher in extreme than both medium and control treatment soils (Table 3-4, Table 3-5).

Table 3-4 Recovered soil carbon (g) from litter in soil at different depths

Depth (cm) \ Rainfall	Control	Medium	Extreme
0-2	0.66	0.59	0.55
2-6	< 0.01	0.03	0.02
6-12	< 0.01	< 0.01	0.04
12-18	< 0.01	< 0.01	0.03
18-25	< 0.01	< 0.01	0.02

Table 3-5 Tukey's test results for comparing litter derived soil carbon at different precipitation regimes

Depth (cm) \ Rainfall	Extreme – Control (g)	Extreme – Medium (g)	Medium – Control (g)
0-2	-0.10 (P = 0.582)	-0.04 (P = 0.936)	-0.07 (P = 0.778)
2-6	**0.02 (P < 0.001)**	< 0.01 (P = 0.568)	**0.02 (P < 0.001)**
6-12	**0.04 (P = 0.001)**	**0.03 (P = 0.004)**	< 0.01 (P = 0.339)
12-18	**0.03 (P < 0.001)**	**0.03 (P < 0.001)**	< 0.01 (P = 0.879)
18-25	**0.01 (P = 0.015)**	**0.01 (P = 0.008)**	< 0.01 (P = 0.820)

Significant differences are indicated in bold (P < 0.05)

3.3.4 Dissolved organic carbon (DOC)

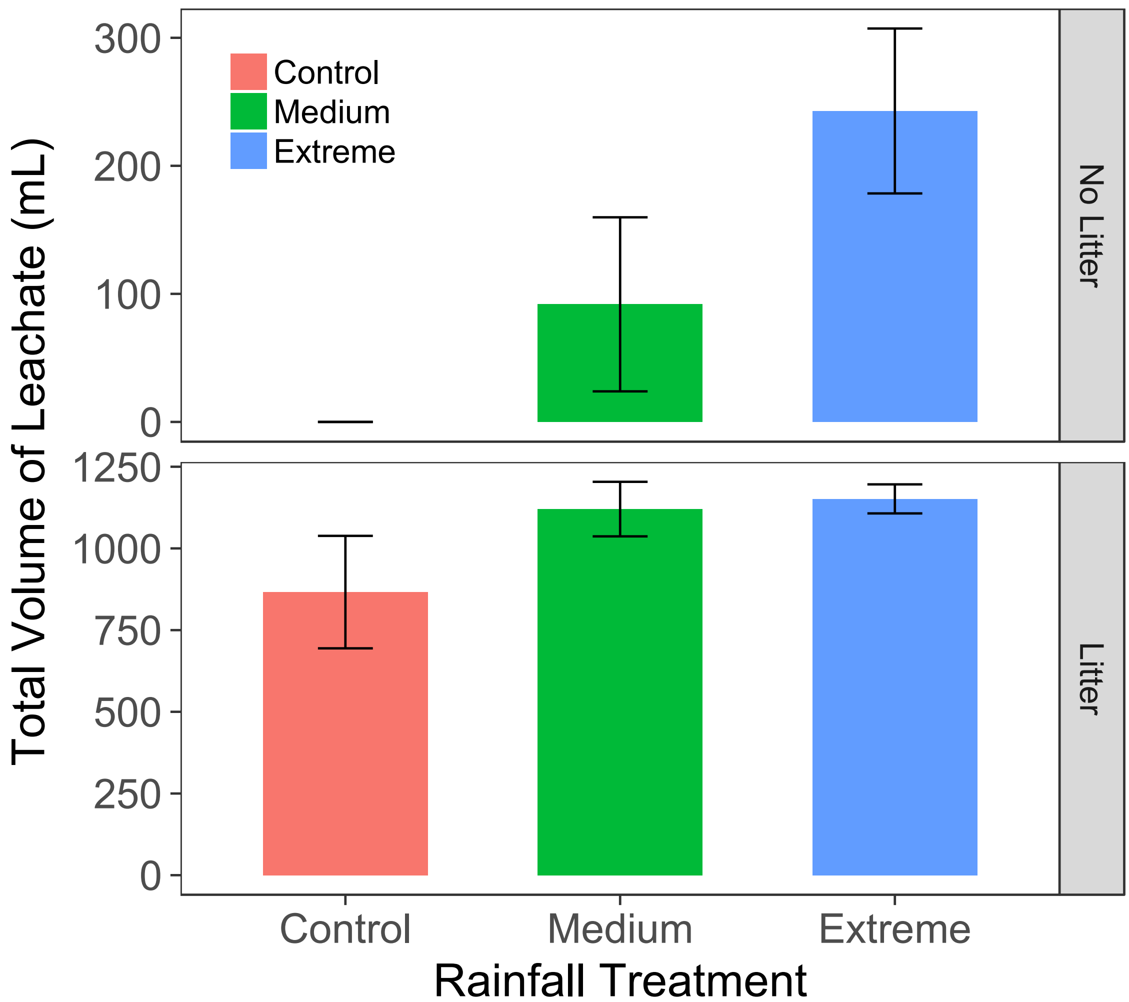

Figure 3-10 Cumulative volume of leachate in different rainfall treatments. Note the different scales in the y axes. In the control rainfall treatment with no litter addition no leachate was collected during the experiment. Two-way ANOVA showed significant effect both for rainfall (p = 0.029) and litter (p < 0.001). Error bars represent standard error.

Total volume of leachate were 0 ml, 91.8 ± 67.9 ml, 243.0 ± 64.4 ml, 866.0 ± 172.0 ml, 1120.0 ± 83.7 ml, and 1152.0 ± 44.3 ml for CNL, MNL, ENL, CL, ML, and EL treatment respectively. Both litter and rainfall effects on total volume were significant, while interaction was not significant. More leachate was collected in litter treatment than no litter treatment ($p < 0.001$), and more leachate was collected in extreme rainfall than control rainfall treatment (p =

0.029) (Figure 3-10). There existed a negative exponential relationship between DOC concentration and volume ($R^2 = 0.63$, $p < 0.001$, Figure 3-11).

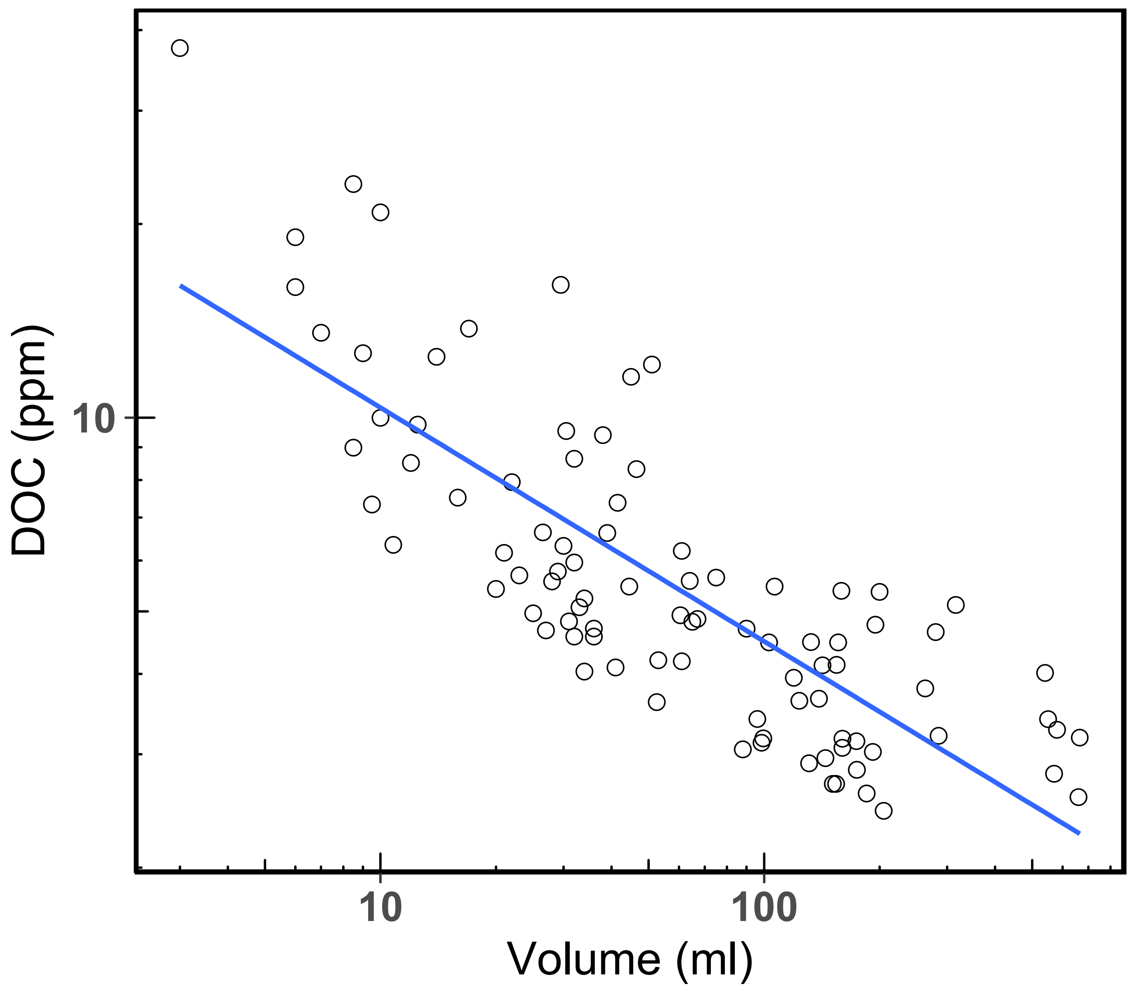

Figure 3-11 Relationship between DOC concentration and volume of leachate ($R^2 = 0.63$, $p < 0.001$). Note both axes are in log scale.

Total carbon leached were 0 mg, 7.5 ± 4.4 mg, 14.0 ± 2.6 mg, 43.8 ± 7.2 mg, 45.9 ± 4.0 mg, and 58.1 ± 6.2 mg for CNL, MNL, ENL, CL, ML, and EL treatment respectively. Similar to volume of leachate, both litter and rainfall effects on carbon leached were significant, while interaction was not significant. More carbon leached in litter treatment than no litter treatment (p < 0.001), and more carbon leached in extreme rainfall than control rainfall treatment (p = 0.026) (Figure 3-12).

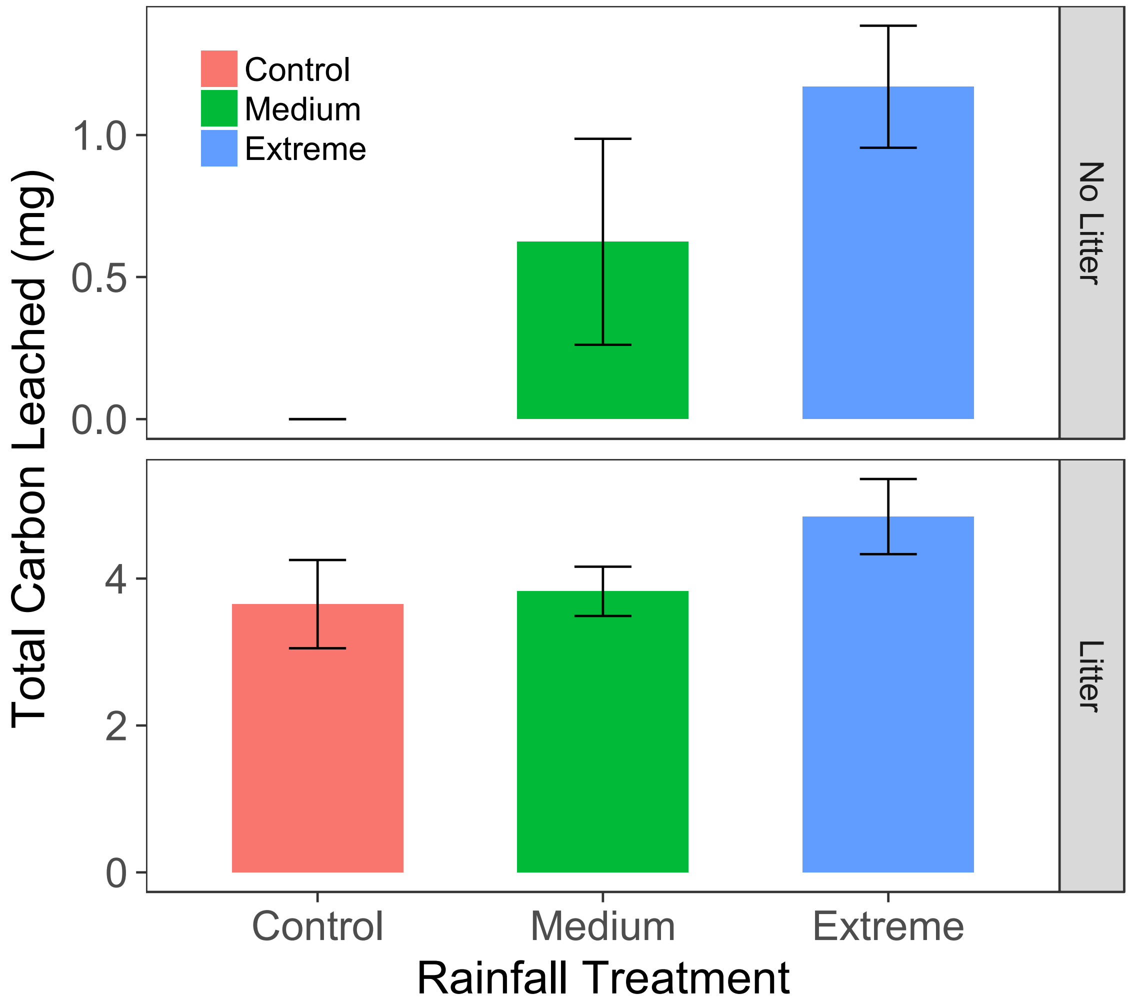

Figure 3-12 Total carbon leached from columns in different treatments. Error bars represent standard error. No carbon leached from control, no litter treatment. Two-way ANOVA shows both rainfall (p = 0.026) and litter (p < 0.001) effects were significant. Error bars represent standard error.

$\delta^{13}C$ of DOC showed different patterns for control and extreme rainfall treatments (Figure 3-13B). In extreme rainfall treatment, $\delta^{13}C$ of DOC was high after first watering event and then quickly decreased, and $\delta^{13}C$ of DOC was consistently higher than base line ($\delta^{13}C$ from no litter treatment soils). In control treatment, at first $\delta^{13}C$ was not different from base line, and as experiment proceeded, $\delta^{13}C$ slowly increased and reached above base line in the end. $\delta^{13}C$ of DOC of medium rainfall treatment was in between extreme and control treatments. Total carbon leached from leaf litter were 0.07 ± 0.03 mg, 0.38 ± 0.14 mg, and 1.87 ± 0.80 mg for CL, ML, and EL treatments respectively. Carbon from litter in extreme rainfall treatment was nearly significantly higher than control treatment ($p = 0.079$, Figure 3-13A).

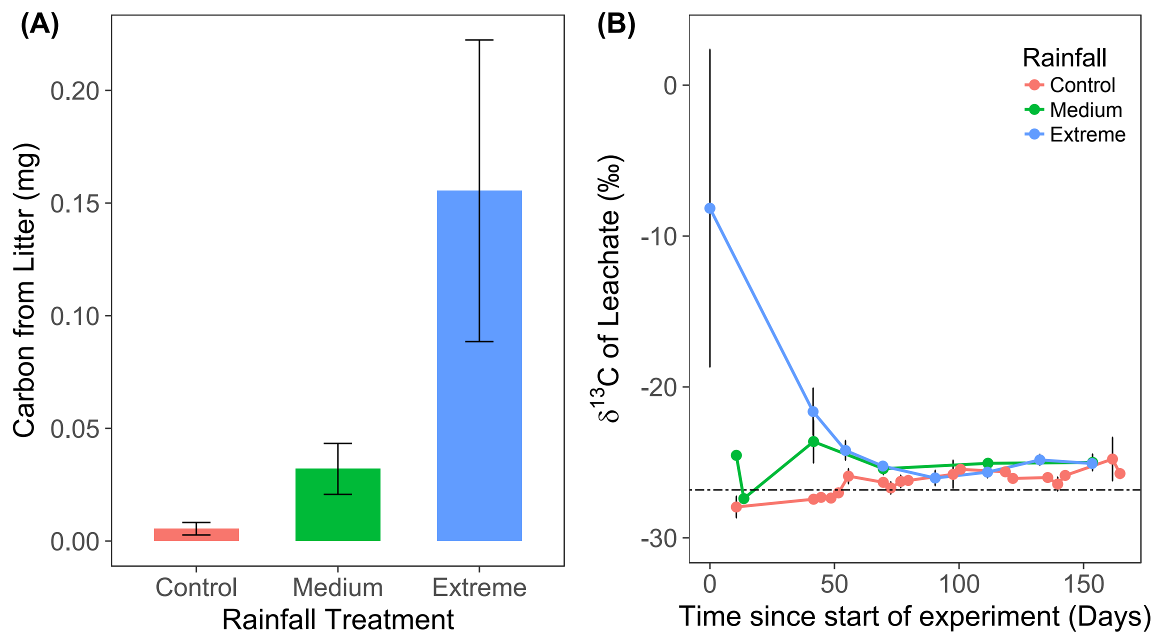

Figure 3-13 Carbon recovered from leaf litter in DOC (N = 3) (A), and temporal change of $\delta^{13}C$ of DOC from litter addition columns in different rainfall treatments (B). Dashed line in plot B represents the mean $\delta^{13}C$ of DOC collected from no litter treatment columns throughout the experiment (N = 8). Error bars represent standard error.

3.4 Discussion

Although there was a trend between rainfall intensity and litter mass loss, the difference was not significant. Thus the results do not support our first hypothesis. However, our second, third, and fourth hypotheses were supported by the data: extreme precipitation resulted in higher carbon content in leachate, although the effect was nearly significant; under extreme precipitation more litter carbon was transported to deeper soil; magnitude of priming effect was smaller in extreme than that in control treatment.

Our partition of litter carbon loss (Figure 3-3) is comparable to other studies in forest ecosystems that mineralization of litter to CO_2 contributed the most, followed by litter carbon transported to mineral soil (Adrian Kammer et al., 2011; Ngao, Epron, Brechet, & Granier, 2005), and leaching of DOC from litter was minimal (Mats Fröberg, Hanson, Trumbore, Swanston, & Todd, 2009; M. Fröberg et al., 2007). Rainfall events directly affect surface decomposition rates by wetting the substrate and stimulating microbial activity. Large fluctuations in precipitation result in extreme wet and dry cycles of the leaf litter. In our experiment, litter mass loss tended to decrease with increasing rainfall intensity. In the control treatment, litter stayed more consistently moist than in the extreme treatment, resulting in an overall higher decomposition rate. Medium treatment lies between control and extreme treatments. Extreme rainfall variability has been tied to slower decomposition of several grassland species (Walter et al., 2013). Drought induced by longer intervals between rainfall events inhibited microbial activity resulting in lower decomposition rate in a temperate grassland (Bloor & Bardgett, 2012).

3.4.1 Litter drove the trend of CO_2 efflux

The sudden pulse-like events of rapidly increasing CO_2 right after rainfall application (Figure 3-4) is a microbial response to increased water and nutrients (W. Borken, Davidson, Savage, Gaudinski, & Trumbore, 2003), and it is referred to as “Birch effect” (Birch, 1964). Litter drove the trend of CO_2 efflux, especially right after watering, which is supported by a rather high proportion of CO_2 coming from leaf litter (Figure 3-5). It was also observed in the field that leaf litter dominated CO_2 pulses after rain and water addition events in a temperate forest

(Cisneros-Dozal, Trumbore, & Hanson, 2007). The high contribution of litter carbon to total CO_2 flux is expected given that in the experimental setup there was no root respiration, and fungal biomass was likely low. Microbial activity is positively related to moisture (Barros, Gomezorellana, Feijoo, & Balsa, 1995) and Lee et al. (2004) found a linear relationship between the contribution of leaf litter to total soil respiration and leaf water moisture in temperate forest. As litter dried out, microbial activity decreased, leading to a decrease of CO_2 efflux and proportion of CO_2 from leaf litter after rainfall events. The decreasing trend of CO_2 efflux and contribution of litter carbon to CO_2 throughout the experiment can be explained by the decrease in labile organic matter and/or by a shift in bacterial community composition (Werner Borken & Matzner, 2009). At a more advanced stage of decomposition leaf litter becomes relatively enriched in recalcitrant chemical components (Bjorn Berg, 2000), resulting in a reduced litter contribution to CO_2 efflux.

3.4.2 More leached DOC and more litter carbon transported to greater depth in extreme treatment

Concentration of DOC negatively correlated with volume of leachate. Due to dilution and less contact time with soil (McDowell & Wood, 1984), a higher volume of water resulted in a lower DOC concentration. Despite the lower [DOC], the greater volume of leachate in extreme rainfall columns yielded more cumulative carbon loss in extreme treatment (Figure 3-12), which agrees with field observations an increase of DOC in forest watersheds under extreme rainfall events (Bernal, Butturini, & Sabater, 2002; Eimers, Buttle, & Watmough, 2008; Hinton, Schiff, & English, 1997).

Although detected ^{13}C signatures in DOC in extreme rainfall treatment, the contribution of litter carbon to DOC was small (Figure 3-13), indicating litter contributes little to DOC compared with soil organic matter in the mineral soil (Mats Fröberg et al., 2009; M. Fröberg et al., 2007). DOC can be retained in soil due to strong abiotic physical-chemical interactions (Frank Hagedorn et al., 2015; Klaus Kaiser & Guggenberger, 2000; McDowell & Likens, 1988) and incorporation of DOC by microbes to form soil organic matter, leading to most litter carbon being retained in 0-2 cm surface soils. While this was the case in our experiment, rainfall intensity clearly affected litter carbon transport supporting our third hypothesis (Figure 3-9A). During extreme events, rapid water movement can bypass precipitation, sorption, and microbial processing, leading to translocation of litter carbon to deeper soil (F. Hagedorn et al., 2000; Klaus Kaiser & Guggenberger, 2005).

Litter carbon transported to deeper horizons can contribute to stable soil organic carbon formation. Subsoil (10-25 cm) has higher clay content, which effectively retains dissolved organic carbon through ligand exchange, hydrogen bonding and/or van der Waals forces (Klaus Kaiser & Guggenberger, 2000; Mikutta et al., 2007). Furthermore, clay-associated soil organic carbon has long residence time (Wattel- Koekkoek, Buurman, van der Plicht, Wattel, & van Breemen, 2003) and is assumed to be responsible for an increased formation of stabilized organic carbon at greater soil depths (Jackson et al., 2017) and in clay rich soils (Müller & Höper, 2004).

The higher $\delta^{13}C$ of DOC in extreme rainfall treatment (Figure 3-13B) agrees with the results that more litter carbon was transported to deeper horizon in extreme than control treatment. However, the high value of $\delta^{13}C$ only happened after the first rainfall event. Litter has more water soluble carbon like sugars, phenolics, and hydrocarbons in the initial phase of decomposition (Bjorn Berg, 2000; B. Berg & Lundmark, 1987), resulting in a high $\delta^{13}C$ of DOC right after the first extreme rainfall treatment. Concentrations of water-soluble substances decreased quickly, leading to a decreasing trend of $\delta^{13}C$ in extreme treatment. However, $\delta^{13}C$ remained above the baseline (mean $\delta^{13}C$ of DOC from no litter treatment soils) throughout the experiment. The source of the litter carbon in the later stages cannot be determined in our setup. It could either be directly leached out from the leaves or from the soil as a result of continuous sorption, precipitation, and microbial processing of litter carbon, followed by subsequent desorption, and dissolution (Klaus Kaiser & Kalbitz, 2012).

3.4.3 Carbon accumulated at surface soil in extreme treatment due to more physical transfer and a lower priming effect

Litter addition only increased soil carbon content at 0-2 cm in extreme treatment. One reason may be more physical transfer of litter particles under extreme rainfall conditions. Extreme rainfall events can break leaf litter into smaller pieces, which then can be transferred more deeply into soil. Physical transfer of litter particulate matter in later stages of decomposition can contribute to SOM formation because of its inherent chemical recalcitrance (M. Francesca Cotrufo et al., 2015).

A lower priming effect in extreme than control rainfall treatment, which supports our fourth hypothesis, is also consistent with our result of a higher carbon content in extreme treatment soils. More litter was decomposed in the control rainfall treatment (though not statistically significant), and less labile carbon was transported into deeper horizon, resulting in litter carbon with more mass and higher quality staying in the surface soil under control than extreme rainfall treatment. Because of the ready availability of soluble organics in the initial phase and accumulation of slowly decomposed litter carbon in later phase, such inputs produced hotspots of microbial activity, which accelerated decomposition of soil organic carbon in control treatment soils (Blagodatskaya, Blagodatsky, Dorodnikov, & Kuzyakov, 2010; Yakov Kuzyakov, 2010; Talhelm, Pregitzer, & Zak, 2009). The delay in detecting the priming effect (Blagodatskaya et al., 2010; Fontaine et al., 2004; Yakov Kuzyakov, 2010) is related to the time needed for DOM to decompose. Even when hydrophilic carbohydrate-rich compounds make up a large fraction of DOM, the decomposition process can take weeks or months (F. Hagedorn & Machwitz, 2007; Kalbitz, Schmerwitz, Schwesig, & Matzner, 2003). Eventually the resources can be exhausted leading to the decline in priming effect (Figure 3-7), although extracellular enzymes produced during the period of high activity remain in the soil (Yakov Kuzyakov, 2010).

3.5 Conclusions

Our ^{13}C tracer experiment clearly shows change of rainfall patterns will alter the relative importance of litter carbon fates. Though contribution of litter carbon to DOC is low due to efficient retention by mineral soil, extreme rainfall events lead to an increase of labile litter carbon in DOC, which may account for the increase of DOC in forest watersheds. We expect that an increase in extreme rainfall events in Northeastern United States will transport litter carbon into deeper horizon in forest, where the labile carbon can be stabilized by interactions with clay minerals, and meanwhile decrease priming effect at soil surface, leading to accumulation of soil carbon at surface. As increases of both extreme rainfall events and amount of precipitation are projected in the future (Trenberth, 2011), litter carbon transport should intensify. Furthermore, in temperate forests, the timing of extreme rainfall events will affect the fate of litter carbon because most litter carbon transport happened at early stages of decomposition, when litter has more water-soluble organic compounds. When soil moisture is not limiting, leaf litter mineralization can still induce an increase of CO_2 efflux after rainfall events. While our lab experiment excluded other important carbon sources and water pathways, such as root respiration and runoff, it still highlights that an increase of extreme events will alter soil carbon cycling processes in temperate forests.

Acknowledgements

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4. Extreme rainfall events transported more litter carbon to deeper horizon during decomposition

Abstract

During decomposition, three pathways contribute to litter carbon loss: carbon dioxide (CO_2), soil organic carbon (SOC), and dissolved organic carbon (DOC). One important factor affecting these processes is soil moisture, which is influenced by precipitation. We conducted a field study to assess the effects of changing precipitation regimes, especially increase of extreme rainfall events, on partitioning of litter carbon. Using ^{13}C labelled tulip poplar leaf litter, we quantified litter decomposition rate and different pathways of litter carbon under different rainfall patterns in a temperate forest in Maryland, USA. Litter decomposition rate was the lowest under extreme rainfall treatment due to temporal drought caused by less frequent rain events. Due to faster water movement during extreme rainfall, more litter-derived carbon was transported to deeper horizon as DOC, and incorporated to form SOC. Additionally, lower initial soil moisture, combined with an extreme rainfall event in the early stage of litter decomposition, translocated more litter carbon to deeper horizon. Our results highlight the importance of rainfall patterns in affecting the partitioning of litter carbon during decomposition. Increase of extreme rainfall events, as projected by most climate models, can result in changes of decomposition rate and litter carbon in DOC and SOC and, eventually may alter soil carbon cycling processes in temperate forests.

4.1 Introduction

Human activities have caused dramatic changes in the global environment, including the increase of greenhouse gas (CO_2, CH_4, N_2O) concentrations due to fossil fuel burning and land use change , which has contributed to the increase of mean annual temperature (IPCC, 2013). Intensification of water cycle, a consequence of global warming, has been expressed in the form of increased drought and heavy precipitation events (Beier et al., 2012; Huntington, 2006). While most general circulation models disagree on the magnitude and even the directional change of rainfall amount at regional and local scales (IPCC, 2013), projections have been consistent in that more extreme rainfall events with longer dry periods in between can be expected in the future (Asadieh & Krakauer, 2015; Trenberth, 2011).

Changes in precipitation, especially increase of extreme rainfall events, lead to changes soil water availability in terrestrial ecosystems. The altered soil water dynamics will affect key ecosystem processes through its influence on plants and soil biota (Beier et al., 2012; A. K. Knapp et al., 2008). One important ecosystem process is litter decomposition, which is a key component in the cycling of terrestrial carbon and other elements.

Litter decomposition rates can be affected by many factors, including climate, litter quality, and the decomposer community composition (Bjorn Berg, 2000; Couteaux et al., 1995; Zhang et al., 2008). Litter decomposition rate generally increases with temperature, precipitation and decrease with lignin content and C:N ratio (Prescott, 2010). Increased leaching, combined with synergistic actions of microbes and soil fauna could explain a higher decomposition rate with increasing precipitation (Salamanca, Kaneko, & Katagiri, 2003). In addition to the total amount, variability of rain events could also affect litter decay. Increased rainfall variability decelerated litter decomposition in a restored prairie due to increased C:N of litter during growth (Schuster, 2016). Consolidating smaller frequent rainfall events to a few heavier precipitation events reduced litter decomposition rate in a temperate forest (Yang et al., submitted).

During decomposition, litter carbon is lost as (1) CO_2 released to the atmosphere, (2) dissolved organic carbon (DOC) leached out of the soil system, and (3) organic compounds translocated into soil to form soil organic carbon (SOC). The application of C isotopes using labeled litter is a useful tool to quantify different litter carbon pathways. Using ^{14}C labeled leaf litter, M. Fröberg et al. (2007) reported a small net transport of DOC from leaf litter, and thus little impact on the carbon stock in the mineral soil below 15 cm. Adrian Kammer et al. (2011) and A. Kammer and Hagedorn (2011) found mineralization of litter to CO_2 was the dominant pathway of litter carbon loss and DOC from leaf litter was mostly retained in the top 5 centimeters of soil using ^{13}C labeled leaves.

Partitioning litter carbon loss during decomposition under different rainfall patterns, especially increase of extreme rainfall events, is essential to understand soil C dynamics in response to future precipitation projections. Numerous rainfall manipulation experiments have been carried out in the past, but most of them were focused on the increase or decrease of total rainfall amount and not on intensity (Beier et al., 2012). Yang et al. (submitted) was the first to explore changing rainfall patterns on partitioning of litter carbon with ^{13}C labeled leaves in the laboratory and found that increase of extreme rainfall events would transport more labile litter carbon to greater depth in the soil profile.

To advance our understanding of the connection between change of rainfall patterns and litter decomposition process and to test whether the laboratory findings apply to field conditions, we designed a litter decomposition experiment under different rainfall patterns in a temperate forest in Maryland, USA. The leaves were ^{13}C labeled to follow different pathways of litter carbon. Total amount of rainfall was kept constant while the frequency and intensity of rainfall events, which were derived from decades of historical precipitation data, were simulated. Each extreme rainfall event was created by consolidating four control rainfall events into one larger event. We hypothesized that (1) litter decomposition rate will be lower under the extreme rainfall treatment, and (2) more litter carbon will be transported to deeper horizons and leach out as DOC in the extreme rainfall treatment.

4.2 Methods

4.2.1 Research site

The Smithsonian Environmental Research Center (SERC) is located along the west coast of Chesapeake Bay in Edgewater, MD (38º53’N, 76º33’W). The major soil types at SERC are Collington sandy loam (fine-loamy mixed, active mesic Typic Hapludult), Annapolis fine sandy loam (fine-loamy, glauconitic, mesic Typic Hapludult), and Donlonton find sandy loam (fine-loamy, glauconitic, mesic Aquic Hapludults) (Yesilonis et al., 2016). The parent material is glauconitic marine sediments lying on the Nanjemoy formation. The forests in this area are a mosaic of stands differing in age, past land use and tree composition. The uncut forest stands were the oldest forest on SERC property and there is no evidence of disturbance such as agriculture and logging. The young forest stands were abandoned from agriculture in the mid-20th century and the old forest stands were abandoned from agriculture or grazing 120-150 years ago (Yesilonis et al., 2016).

The mean precipitation in the region is 114.6 cm and the mean annual temperature is 13 °C (D.Correll, T. Jordan, and J. Duls, unpublished data). Data from a meteorology tower at SERC showed that in 2017-2018 daily maximum air temperature varied from -7.7 °C to 34.2 °C.

Total precipitation was 786 mm in 2017 and 1074 mm in 2018 from 1-1-2018 to 9-12-2018 (**Error! Reference source not found.**), with 2018 being one of the wettest years in history.

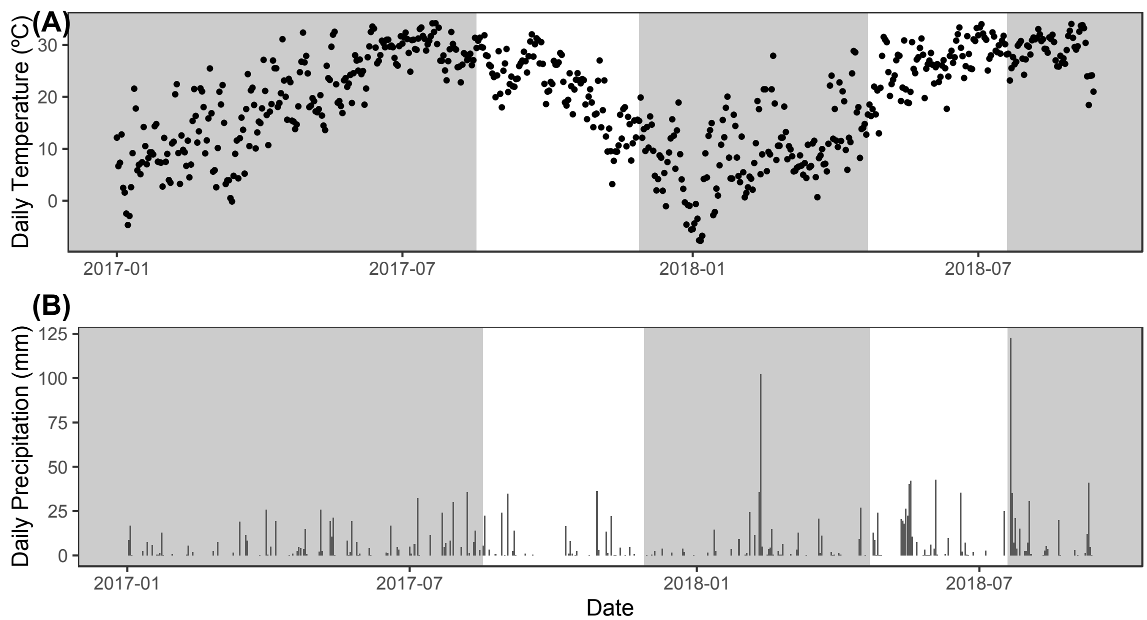

Figure 4-1 Daily maximum temperature (A) and daily precipitation (B) from a meteorology tower at Smithsonian Environmental Research Center (SERC). The white area represents the time during which we manipulated rainfall patterns and measured CO_2 flux.

Our study site is one of the old forest stands dominated by tulip poplar (*Liriodendron tulipifera*), sweet gum (*Liquidambar styraciflua*), oaks (*Quercus spp*.), American beech (*Fagus grandifolia*), and hickories (*Carya spp*.). The Bt horizon to the surface is around 25 cm and the pH at surface soil (0-10 cm) is 4.9 (Yesilonis et al., 2016). At this site there is little persistent forest floor and the O horizon is very thin because of the presence of earthworms, both native and non-native (Szlavecz et al., 2018).

4.2.2 Historical precipitation data in SERC to determine control and extreme rainfall treatments

The three rainfall treatments established in the field were based on historical precipitation data from SERC and surrounding weather stations. Rainfall intensity was derived from 15-minute precipitation data from US Custom House in Baltimore (1984 – 1999) (https://www.ncdc.noaa.gov/cdo-web/datatools). Frequency of heavy rainfall events was derived from daily precipitation data from US Naval Academy (1894 – 1976) (https://www.ncdc.noaa.gov/cdo-web/datatools) in Annapolis, Maryland and from SERC weather station data (2002 – 2013). A comparison of historical precipitation and manipulated rainfall patterns is presented in Yang et al., (submitted). Both rainfall frequency and intensity were manipulated, while total rainfall remained constant. The intensity and frequency of rainfall events in the control treatment were mean of 15-minute rainfall intensity and mean frequency of rainy days, respectively. In the extreme treatment, the intensity and frequency were top 1% of 15-minute rainfall intensity and top 1% of frequency of rainy days respectively. The medium rainfall treatment was alternating between control and extreme rainfall treatments in a two-week interval. As a result, during a four-week period, the extreme treatments received two heavy rainfall events, the control treatments received eight rainfall events with low intensity, and the medium treatments received four rainfall events with low intensity in the first two weeks, followed by one heavy rainfall event. A more detailed description is provided by Yang et al., (submitted).

4.2.3 Field experiment setup

A modified version of the rainout shelter by Yahdjian and Sala (2002) has been built to control rainfall intensity and frequency in the forest. Three subplots within distance of 20 meters were chosen in our site and three rainout shelters, one for each treatment, were built in each subplot. Each shelter is 2m × 2m with 1m and 1.7m height in front and back, respectively. Transparent roof was used to exclude rain and channel water to gutters and rain barrels. Under each rainout shelter an area of 1.2m by 1.2m was established as watering area and split in half with plastic garden edging. One half was covered with leaf litter, while the on the other half the soil remained bare throughout the experimental period.

Under each rainout shelter, four PVC rings (diameter: 15.2 cm) were permanently inserted into the soil in the middle of the watering area to avoid edge effects. Two of the rings received ^{13}C labeled tulip poplar (*L. tulipifera*); while the other two did not. ^{13}C enriched tulip poplar (*L. tulipifera*) leaf litter was produced in an enrichment chamber as described in Bernard et al. (2015). To ensure homogeneity of the litter substrate, prior to adding leaf litter to the plots, petioles were removed, dry leaves were broken up into smaller pieces and sieved through an 8-mm sieve. Leaf litter was placed into litterbags (0.8 mm hole size) to prevent direct consumption by arthropods and earthworms. Empty litterbags were placed in bare soil rings.

To monitor environmental conditions 10 HS soil moisture smart sensors (S-SMD-M005, Onset, Bourne, MA, USA) and HOBO 2x external temperature data loggers (U23-003, Onset, Bourne, MA, USA) were installed in two of the three subplots at 10 cm depth. Moisture and temperature sensors took a reading at every 10 and 30 minutes, respectively. In 2017, soil moisture sensors were only installed under litter treatment soils in one of the two subplots that had Onset sensors installed. The one subplot without Onset sensors was instrumented with ECH$_2$O EC-5 moisture sensors (METER Group, Pullman, Washington, USA) and a MULTI-THERMO digital thermometer at 10 cm depth to measure soil moisture and temperature when taking CO_2 measurements. To collect leachate and to quantify dissolved organic carbon, suction soil water samplers (1900L24-B02M2, Soilmoisture Equipment Corp, Goleta, CA, USA) were installed at 30 cm depth at an angle of 45° to the soil surface. Prior to each rainfall event, a vacuum of 60 centibars was applied to create a vacuum environment. Leachate was then collected using the samplers after rainfall events. All leachate collected under each ring during a two-week period were consolidated into one leachate sample. Thirty milliliter subsamples were then passed through a 0.45µm glass fiber filter, acidified with phosphoric acid, and stored at 4 °C for carbon concentration and isotope analysis.

In August 2017, 4.2 g tulip poplar leaves were put into each of the bags, an amount that is similar to tulip poplar litterfall input in old forest stands at SERC (Szlavecz et al., 2018). There were two leaf litter manipulations, and three rainfall manipulations, control, medium, and extreme, with six replicates in each combination. The six treatments hereafter are labeled as CL (control-litter), CNL (control-no litter), ML (medium-litter), MNL (medium-no litter), EL (extreme-litter), and ENL (extreme-no litter). Rainfall treatments were manipulated by manually spraying deionized water with a watering can as evenly as possible in 15-minute intervals. For the control rainfall treatment, 790 mL water was applied to half of the shelter every 15 minutes for 2.5 hours. For the extreme rainfall treatment, 5265 mL water was applied to half of the shelter every 15 minutes for 1.5 hours. In 2017, treatment started in August and ended in November, with each shelter watered for 12 weeks. The remaining leaves were collected, dried and stored in the lab to avoid disturbance during winter when no CO_2 measurements were taken. In 2018 we added an additional 4.2 g tulip poplar litter to the bags before putting them back in April. The rainfall treatments started again in April and ended in July, with each shelter watered for another 12 weeks. In May 16-18 the region was hit by torrential rains (104.6 mm) saturating the soil and threatening the experimental setup to be washed away. During this time the litterbags were picked up, stored in the laboratory and returned the following week.

4.2.4 CO_2 flux measurement

CO_2 flux was measured at least weekly, and more frequently before and after rainfall events. The static chamber method was used to determine CO_2 fluxes. PVC collars were permanently installed into soil to measure soil CO_2 efflux. A PVC chamber assembled with a CO_2 sensor (GMP 343 Vaisala, Finland) was placed on the collar while measurements were taken. CO_2 concentrations in the headspace were recorded every second for 6 minutes. CO_2 efflux, F, can be calculated as:

$$F = \frac{dC}{dT} * \frac{V}{S}$$

where F is the gas flux in µmol m^{-2} s^{-1}, C is the mole concentration in µmol m^{-3}, T is time, V is the volume of headspace, and S is the area of the soil surface in the chamber. dC/dT can be approximated as slopes of fitted lines between CO_2 concentration and time. The coefficient (R^2) of determination values of these fitted lines were usually larger than 0.95. CO_2 measurements usually took place from 10:00 am to 6:00 pm Eastern Standard Time.

4.2.5 $\delta^{13}C$ of respired CO_2 measurements

$\delta^{13}C$ of respired CO_2 was measured approximate monthly from September to November in 2017 and from May to July in 2018 for a total of six campaigns. When taking $\delta^{13}C$ measurements, a total of four gas samples of 60 ml were collected at different CO_2 concentrations using Cali-5-Bond air & gas sampling bags (Calibrated Instruments Inc., McHenry, Maryland, USA). CO_2 concentration and $\delta^{13}C$ of CO_2 was determined by a cavity ring-down spectroscopic carbon isotope analyzer (Picarro G2101-i, Picarro Inc., Santa Clara, California, USA) connected to an automated sampling manifold (Picarro A0311). We used keeling plots to calculate the $\delta^{13}C$ of respired CO_2 (Brand & Coplen, 2012). We occasionally dropped a data point because of poor quality data from the analyzer. For each case, the slope was based on ≥ 3 observations. For a slope to be determined as a quality data point, the R^2 had to be greater than 0.75.

To gain insight on how the $\delta^{13}C$ of respired CO_2 from leaf litter would change after rainfall events, several time points were selected for gas sampling in both control and extreme rainfall in litter addition treatments for two of the shelters. In general, $\delta^{13}C$ of respired CO_2 was measured immediately after (within 30 minutes, T_{C1}), 1 day after (T_{C2}), and 3.5 days after (T_{C3}) control shelter received a rainfall treatment. While for extreme rainfall treatment, $\delta^{13}C$ of respired CO_2 was measured immediately after (within 30 minutes, T_{E1}), 2 days after (T_{E2}), 1 week after (T_{E3}), and 2 weeks after (T_{E4}) rainfall event.

4.2.6 Litter collection and soil sampling

At the conclusion of the experiment, all litter bags were collected and a composite sample of three cores inside each ring was collected. Cores were divided into five depths: 0-2, 2-5, 5-10, 10-20, and 20-30 cm. The collected litter residues and soil samples were oven dried at 70 °C to constant weight and ground for later isotope analyses.

4.2.7 Stable isotope analysis

The C elemental and stable isotope composition of leaf litter, soil, and leachate samples were analyzed at the UC Davis Stable Isotope Facility (Davis, California, USA). Leaf litter was analyzed using a PDZ Europa AnCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Soils were analyzed using an Elementar Vario EL Cube or Micro Cube elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) coupled with a PDZ Europa 20-20 isotope ratio mass spectrometer. Dissolved organic carbon of leachates was analyzed for ^{13}C using an O.I. Analytical Model 1030 TOC Analyzer (Xylem Analytics, College Station, TX) interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer utilizing a GD-100 Gas Trap Interface (Garden Instruments).

Stable isotope ratios of C ($^{13}C/^{12}C$) are expressed using delta (δ) notation: $\delta^{13}C = [R_{sam}/R_{std} - 1] \times 1000‰$, where R_{sam} is the isotope ratio ($^{13}C/^{12}C$) in the samples, and R_{std} is the isotope ration in the standard, which is Vienna Pee Dee Belemnite for C.

4.2.8 Leachate-C calculations

The fraction of leachate derived from litter f_l can be estimated by applying a two source mixing model (Balesdent et al., 1987):

$$f_l = \frac{\delta_{s-l} - \delta_{n-l}}{\delta_l - \delta_{n-l}}$$

where δ_{s-l} is $\delta^{13}C$ of DOC from litter treatment soils, δ_{n-l} is $\delta^{13}C$ of DOC from no litter treatment soils, and δ_l is the $\delta^{13}C$ value of the litter sample. We assumed that the $\delta^{13}C$ values of the litter-derived leachate are equivalent to the $\delta^{13}C$ values of the bulk litter. The amount of carbon from leaf litter in DOC was calculated as the sum of litter carbon in leachate M_{l-l}:

$$M_{l-l} = V \times C_{carbon-l} \times f_l$$

where V is volume of leachate, and $C_{carbon-l}$ is the concentration of DOC.

4.2.9 Soil moisture sensor readings correction

Due to torrential rains in mid-May 2018, one soil moisture sensor under litter treatment were unstable, producing wildly-fluctuated and erroneous values. To correct for the readings, we first calculated the difference of soil water content between litter and no litter treatment (litter – no litter), and then established a difference value bigger than 0.06 to be incorrect. The difference of soil water content was modeled as a function of time using natural cubic splines to capture the trend of difference. We then applied the model for the time points when we had incorrect readings to calculate the expected difference and added soil water content in no litter treatment to get the correct readings.

4.2.10 Statistical Analysis

All statistical analyses were conducted using R version 3.3.3. P values below 0.05 were considered significant and those between 0.05 and 0.1 were considered nearly significant. Litter effect on soil temperature at 10 cm depth was evaluated by calculating hourly average of soil temperature data from all six shelters with Onset sensors installed under litter vs. no litter treatment from August 3rd 2017 to August 6th 2018. The difference of soil temperature between no litter and litter (no litter – litter) treatment was also calculated and averaged over different hours and months to produce contour plot to explore the temporal change of the difference using the same soil temperature dataset. One-way analysis of variance (ANOVA) followed by Tukey's HSD for multiple comparisons was used to assess the effect of rainfall treatment on litter mass loss. Linear mixed models were run to test effects of year, litter, rainfall and year×rainfall on total leached carbon and carbon leached from litter, effects of weeks, year, rainfall and weeks×rainfall on $\delta^{13}C$ of DOC at 30 cm depth and on $\delta^{13}C$ of respired CO_2 right after rainfall from litter treatment soils. Ring ID was treated as random effect and fixed effects were evaluated in the models following the order previously mentioned. Likelihood ratio tests were used to assess significant differences between nested models, and were followed by Tukey's HSD test for multiple comparisons using *multicomp* and *emmeans* packages. To evaluate the effect of soil moisture on leachate volume, we first calculated the average soil moisture during the two-week period and then evaluated the effect using linear regression. Litter effect on $\delta^{13}C$ and carbon content at different depths was evaluated using t-tests. If the t-test was significant, differences were calculated (litter – no litter) and effect of rainfall was assessed using either t-test (two treatments) or one-way ANOVA (three treatments) followed by Tukey's HSD test.

4.3 Results

4.3.1 Soil temperature and soil moisture under the shelters

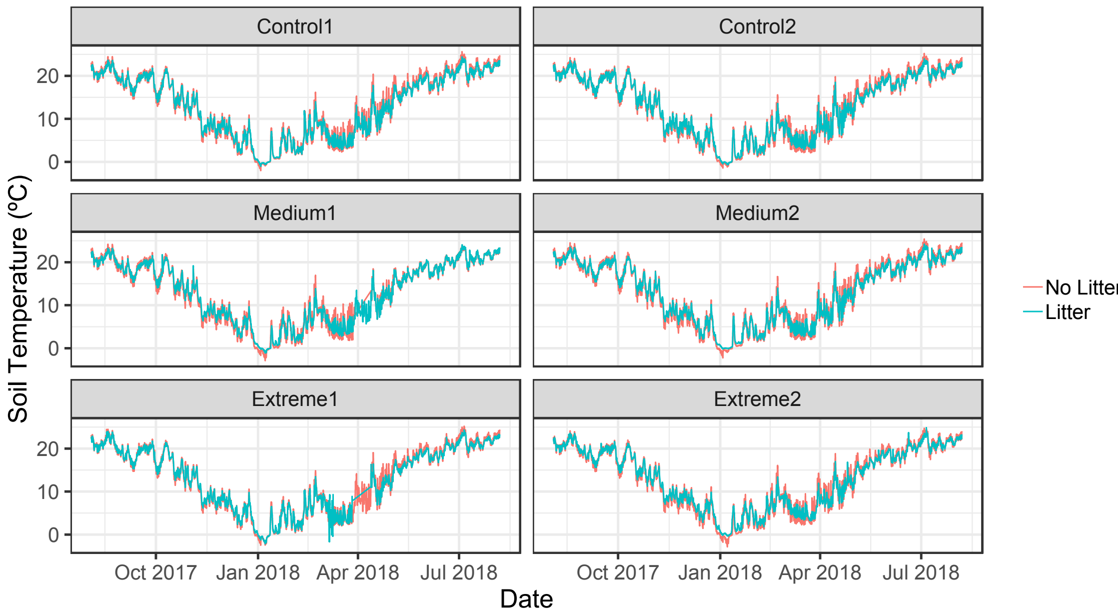

Figure 4-2 Soil temperature at 10 cm depth in litter vs. no litter treatment soils under different rainfall treatments from August 2017 to August 2018. Two of the three subplots were equipped with the sensors.

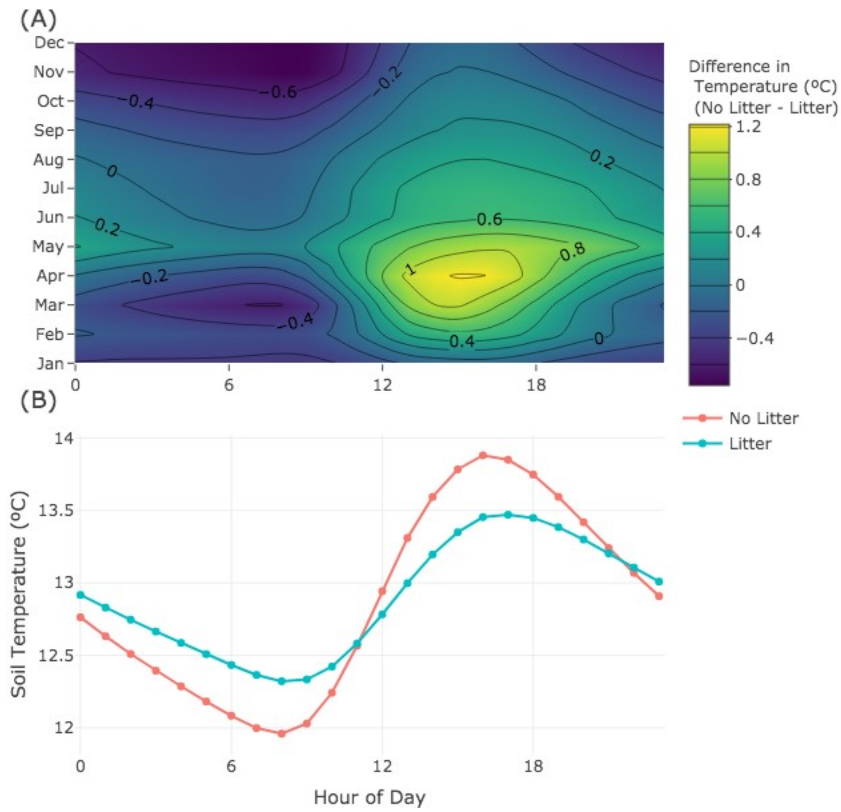

Figure 4-3 (A) Contours of difference of soil temperature at 10 cm depth (no litter – litter) as a function of hour of day and month and (B) diurnal average soil temperature at 10 cm depth in litter vs. no litter treatment soils. Hourly mean soil temperature was calculated from all shelters equipped with Onset sensors from August 3, 2017 to August 6, 2018. The same dataset was used to calculate monthly average soil temperature difference and to generate the contour plot.

Soil temperature ranged from -2.9 °C to 25.6 °C (Figure 4-2). In general, both soil temperature and soil temperature difference (litter – no litter) at 10 cm depth were the lowest at 7:00-8:00 am and highest at 3:00-4:00 pm (Figure 4-3). Litter acted as an insulation layer and dampened daily variation of soil temperature: soil was cooler during daytime and warmer during nighttime under litter than no litter treatment soils. However, the pattern of diurnal soil temperature difference varied throughout the year (Figure 4-3A). Between January and April, the difference of soil temperature followed the typical pattern. Once canopy cover developed, preventing direct sunlight to the soil surface, the temperature difference decreased. From late

November to early January, the coldest time of the year, soil temperature difference reversed during daytime: litter treatment soils were also warmer than no litter treatment soils at daytime.

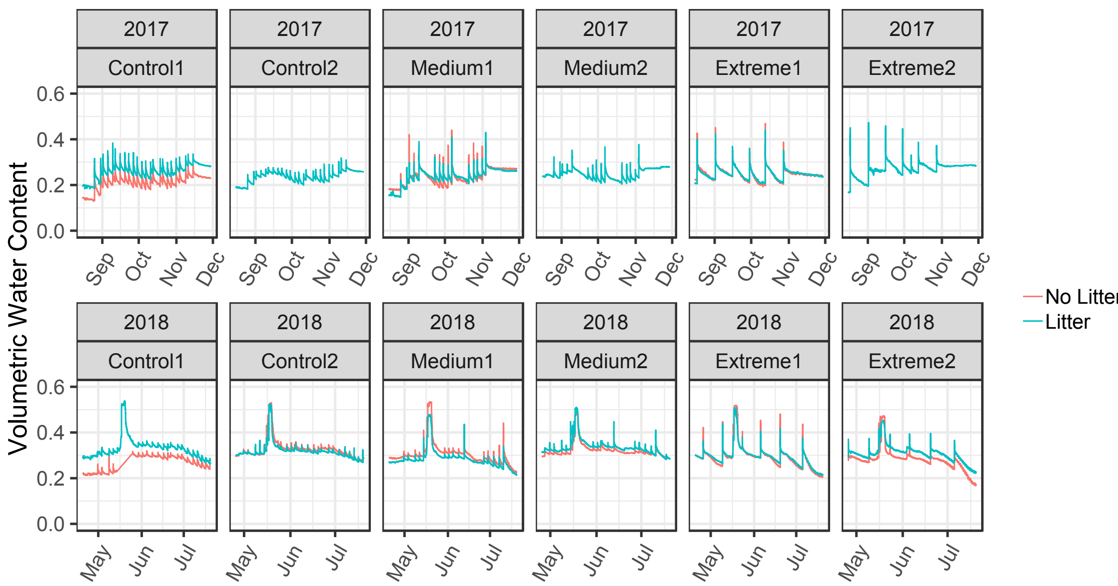

Figure 4-4 Volumetric water content at 10 cm depth in litter vs. no litter treatment soils under different rainfall treatments during the period when we manipulated rainfall patterns. In 2017 one subplot was equipped with sensors in both litter and no litter treatments, the other subplot had sensors in the litter treatment half. In 2018 two of the three subplots had sensors in both halves. Peaks of soil moisture indicate rainfall events except in 2018 mid-May. A three-day long torrential rain saturated the soil. Note that 2017 data indicate fall-winter, whereas 2018 values were taken late spring- summer.

Generally, litter layer reduced evaporation, leading to higher soil moisture in litter than no litter treatment in most subplots (Figure 4-4). Peaks of soil moisture corresponded to rainfall treatments and the peak was higher in extreme than control rainfall treatments. There existed an extra peak in our site at around May 18th when soils became saturated due to consecutive rainfall of 104.6 mm rainfall from May 16th to May 18th. Year 2018 was one of the wettest years for Maryland, with May-July period being the wettest in state history (https://www.climate.gov/news-features/event-tracker/soggy-summer-mid-atlantic-2018), resulting in higher soil moisture in 2018 than 2017 most of the time. A decreasing trend of soil moisture in summer 2018 indicates water uptake by trees.

4.3.2 Litter mass loss

During the six months of the experiment, tulip poplar leaf litter lost 46.5 ± 0.5% mass in control rainfall treatment, 43.5 ± 1.1% in medium rainfall treatment, and 39.1 ± 0.8 % in extreme rainfall treatment (Figure 4-5). Mass loss was significantly less in extreme rainfall than

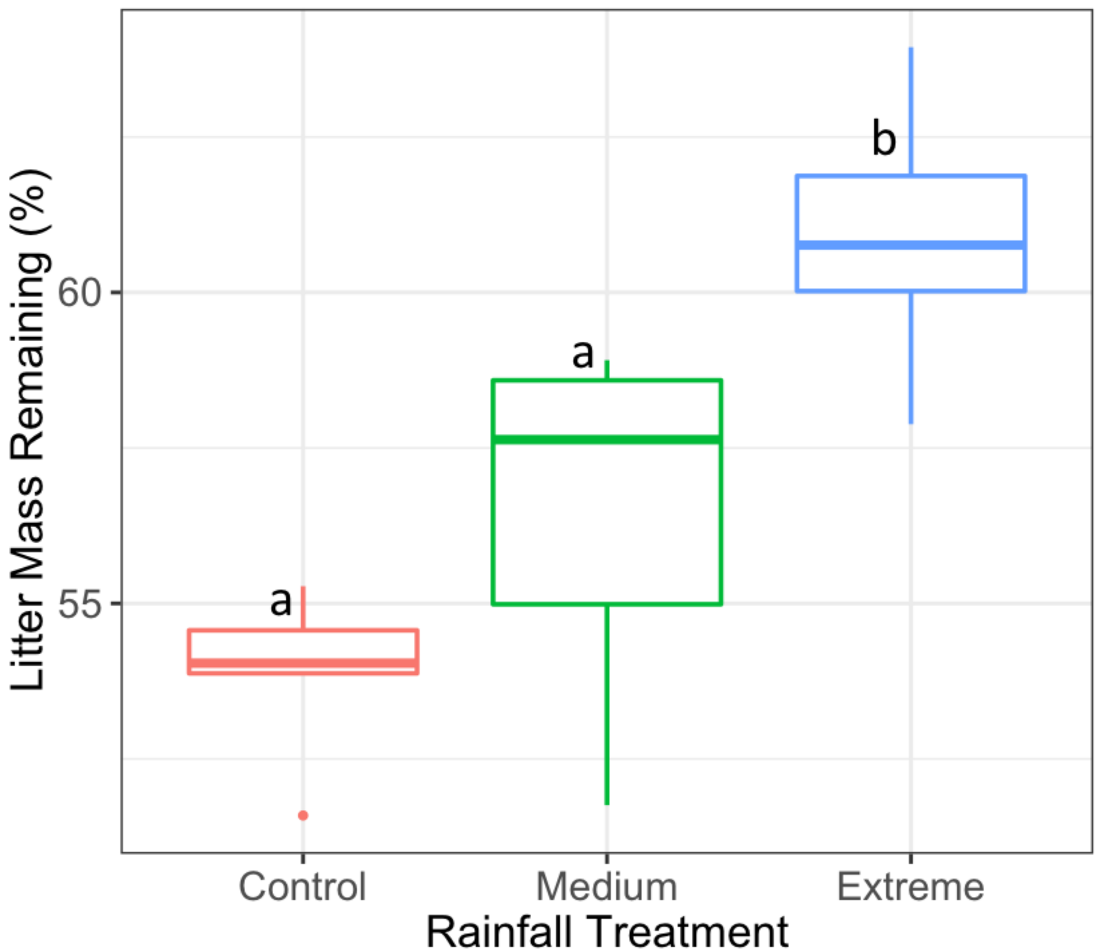

Figure 4-5 Litter mass remaining in different rainfall treatments. Boxes with different letters are significantly different (Tukey's HSD, $P < 0.05$).

both medium ($p = 0.008$) and control ($p < 0.001$) treatments while no significant difference was detected between medium and control treatments.

4.3.3 CO_2 efflux

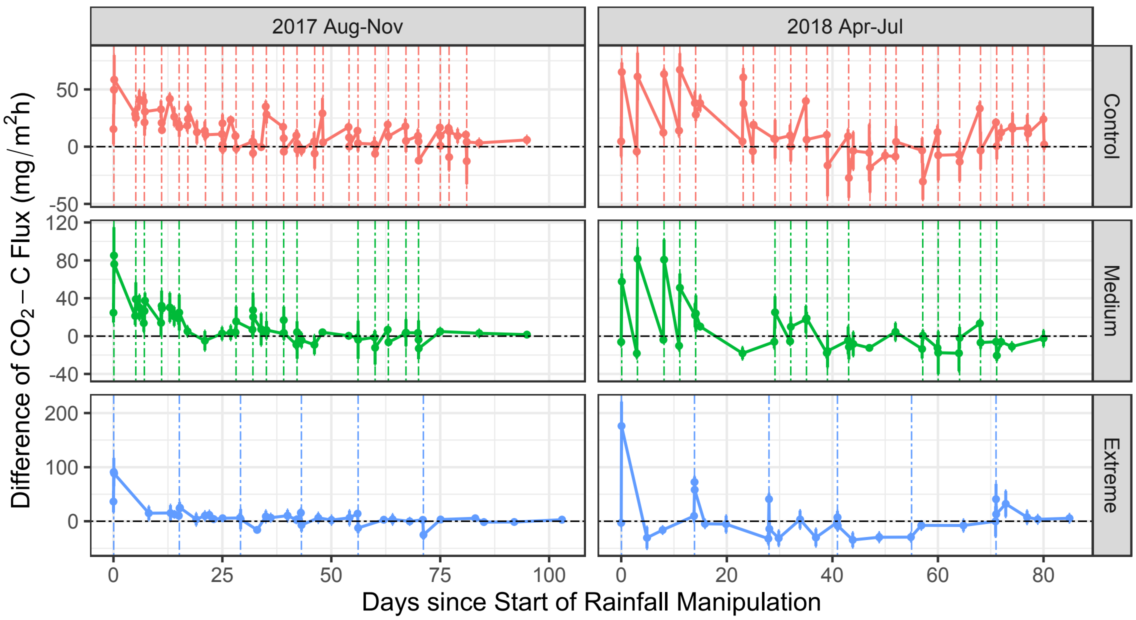

Figure 4-6 Differences in CO_2 efflux (litter – no litter) under different rainfall treatments. Mean values and standard error are shown (N = 3). Horizontal dashed lines indicate no difference in CO_2 efflux, while vertical dashed lines represent rainfall manipulation events.

The difference in CO_2 efflux (litter – no litter) was the highest right after rainfall manipulation in all treatments, especially in the beginning of each year, and quickly dropped afterwards (Figure 4-6). The difference in efflux exhibited a decreasing trend over the course of our experiment in each year, and even became negative in the later phase.

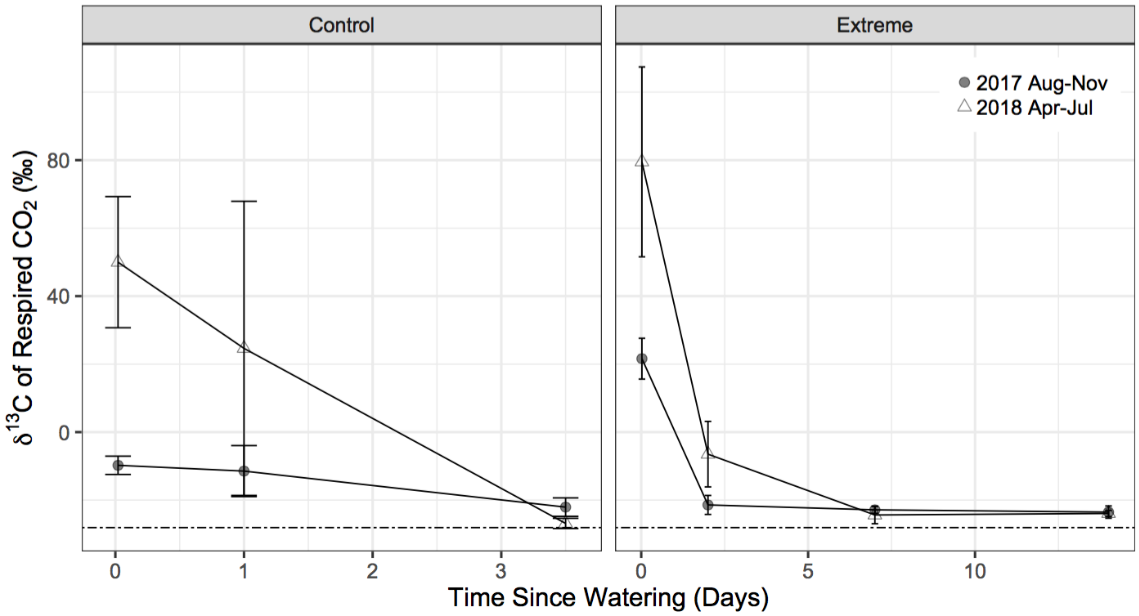

Figure 4-7 Temporal change of $\delta^{13}C$ of respired CO_2 under control and extreme rainfall treatments from soils with litter. Note x axes are in different scales. Error bars represent standard error. Dashed lines represent $\delta^{13}C$ of respired CO_2 from soils without litter addition.

Consistent with the difference in total CO_2 efflux, right after each rainfall event, the proportion of litter derived CO_2 increased and then decreased very quickly (Figure 4-7), as evidenced by the temporal change of $\delta^{13}C$ of respired CO_2. We used linear mixed models to examine the effect of rainfall on the $\delta^{13}C$ of CO_2 right after rainfall treatments (T_{C1} vs. T_{E1}) and found $\delta^{13}C$ of respired CO_2 was higher in 2018 than 2017 ($p = 0.002$), and nearly higher in extreme than control rainfall treatment ($p = 0.051$) (Table 4-1).

Table 4-1 Results of mixed effect models testing the effects of weeks, year, rainfall and weeks × rainfall on $\delta^{13}C$ of respired CO_2 right after rainfall from litter treatment soils and $\delta^{13}C$ of DOC at 30 cm depth

	Weeks		Year		Rainfall		Weeks × Rainfall	
	χ^2	P	χ^2	P	χ^2	P	χ^2	P
$^{13}CO_2$	10.8	0.004	9.4	0.002	3.8	0.051	4.1	0.13
$DO^{13}C$	15.6	0.008	7.6	0.006	3.6	0.162	22.8	0.012

4.3.4 Dissolved organic carbon (DOC)

More volume of leachate was collected in 2018 than 2017 (Table 4-2, Table 4-3). The effect of adding litter was non-significant. Rainfall × Year interaction was significant: in 2017, more leachate was collected in extreme than control ($p = 0.013$); in 2018, rainfall effect was not significant (Table 4-3). Linear positive relationships were found between volume of leachate collected and average soil moisture during two-week intervals in all rainfall treatments (Figure 4-8).

Table 4-2 Total volume of leachates and total carbon leached at 30 cm under different rainfall treatments in years 2017 and 2018 (N = 2). CL: Control-Litter, CNL: Control-No Litter, ML: Medium-Litter, MNL: Medium-No Litter, EL: Extreme-Litter, ENL: Extreme-No Litter

Treatment	CL		CNL		ML		MNL		EL		ENL	
Year	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018
Volume (ml)	466	5734	354	5590	952	5646	838	5432	2176	4788	1858	3341
Carbon (mg)	1.41	13.80	1.36	12.90	2.69	11.40	3.00	14.30	9.76	10.50	5.49	7.28

Table 4-3 Results of mixed effect models testing the effects of weeks, year, litter, rainfall and year×rainfall on leachate volume

	Weeks		Year		Litter		Rainfall		Year×Rainfall	
	χ^2	P	χ^2	P	χ^2	P	χ^2	P	χ^2	P
Leachate	16.1	0.006	106.3	< 0.001	0.5	0.487	0.7	0.701	23.4	< 0.001

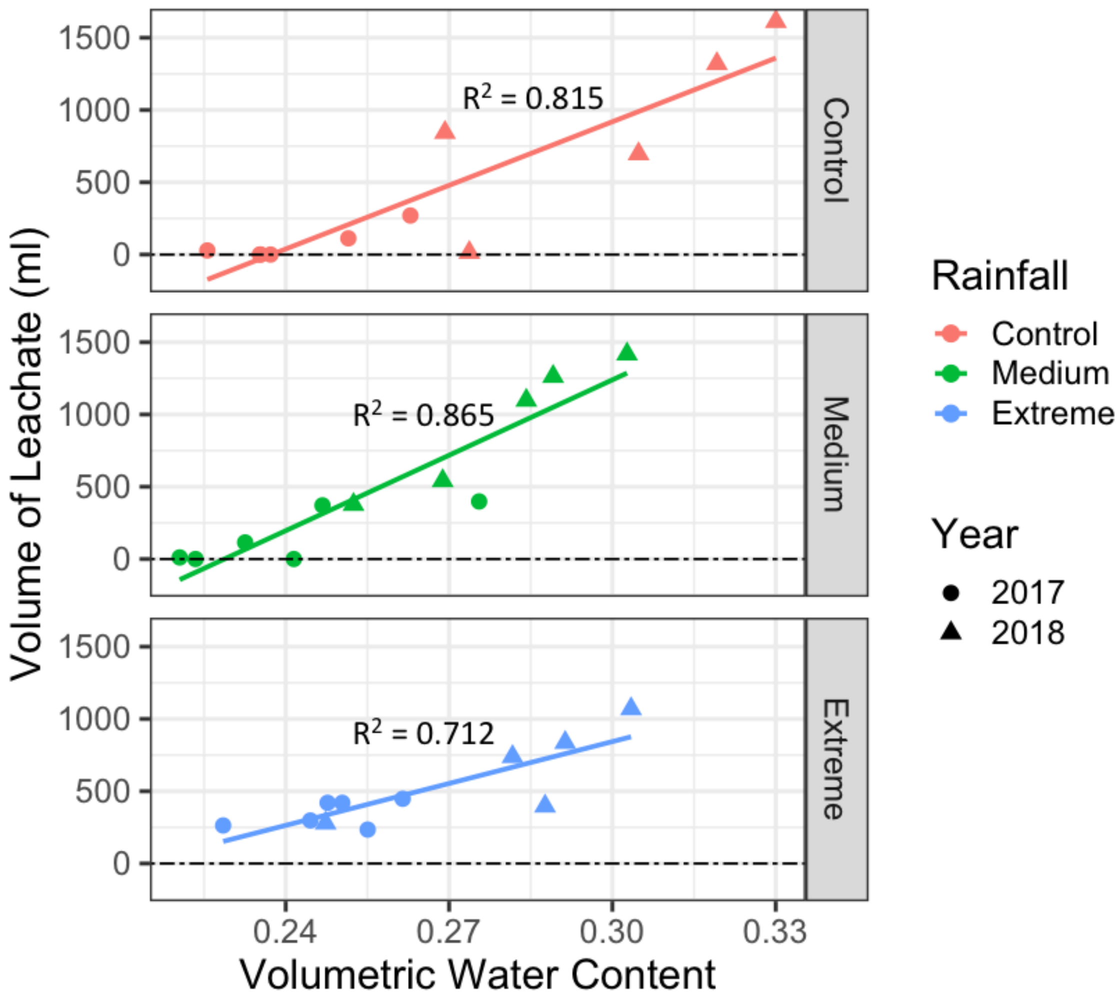

Figure 4-8 Relationship between volume of leachate collected and average soil moisture during two-week intervals for different rainfall treatments. R^2 values came from fitting linear regression models separately for different treatments.

Similar to average volume of leachate, more total carbon was leached in 2018 than 2017 in control and medium rainfall treatments (Figure 4-9). Rainfall × Year interaction was significant: in 2017, more carbon was leached in extreme than control ($p = 0.017$); in 2018, rainfall effect was not significant (Figure 4-9, Table 4-4).

Table 4-4 Results of mixed effect models testing the effects of year, litter, rainfall and year × rainfall on total leached carbon (TC) and carbon leached from litter (LC)

	Year		Litter		Rainfall		Year × Rainfall	
	χ^2	P	χ^2	P	χ^2	P	χ^2	P
TC	23.2	< 0.001	0.2	0.697	1.0	0.593	17.6	< 0.001

LC	1.9	0.173	NA	NA	6.1	0.047	8.7	0.013

NA: not applicable

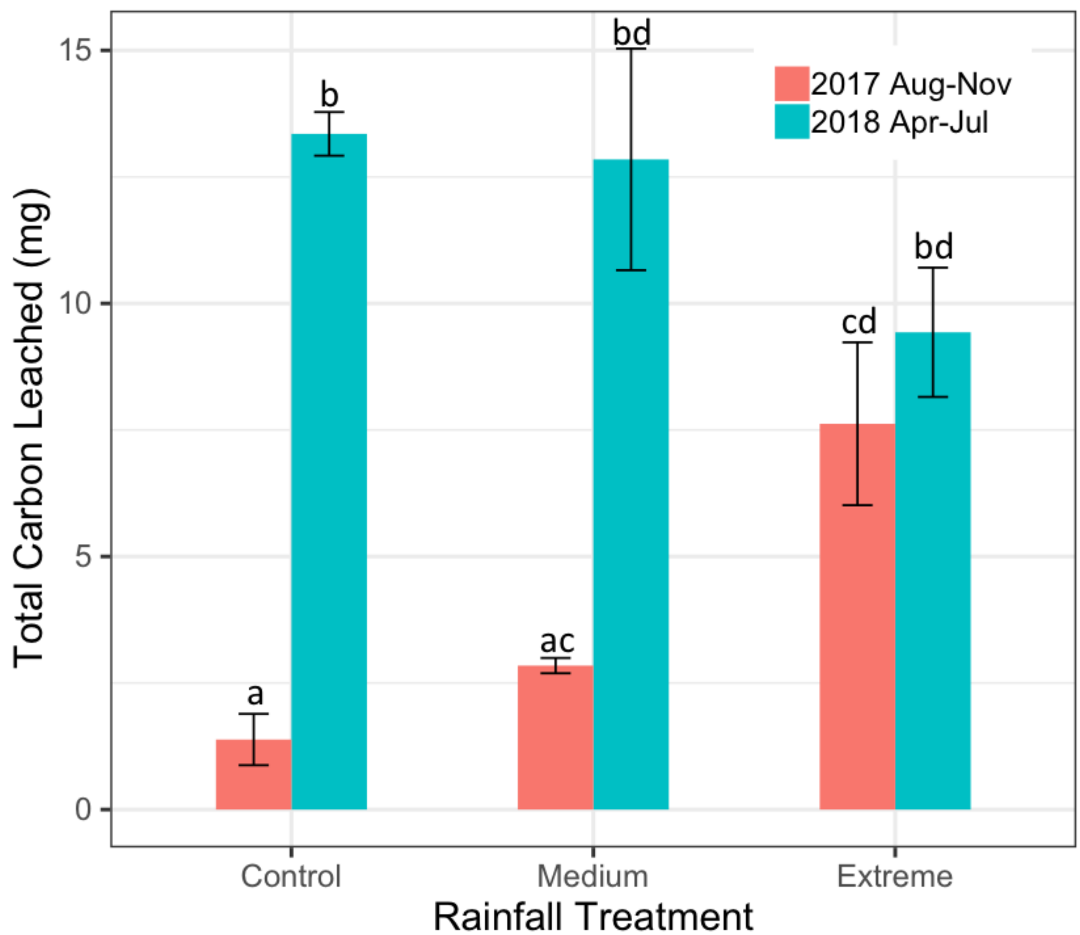

Figure 4-9 Total leached carbon as DOC in different rainfall treatments. Means with different letters are significantly different (Tukey's HSD, P < 0.05). Error bars represent standard error.

Litter derived carbon as DOC were 0.006 ± 0.006, 0.025 ± 0.003, and 0.640 ± 0.329 mg for CL, ML, and EL in 2017, and 0.005 ± 0.004, 0.027 ± 0.002, and 0.068 ± 0.042 mg for CL, ML, and EL in 2018 (Figure 4-10B). Rainfall × Year interaction was significant: in 2017, more litter derived carbon was leached as DOC in extreme than both control (p = 0.032) and medium (p = 0.040); in 2018, rainfall effect was not significant; more litter derived carbon was leached in 2017 than 2018 under extreme treatment (p = 0.042) (Figure 4-10B, Table 4-4).

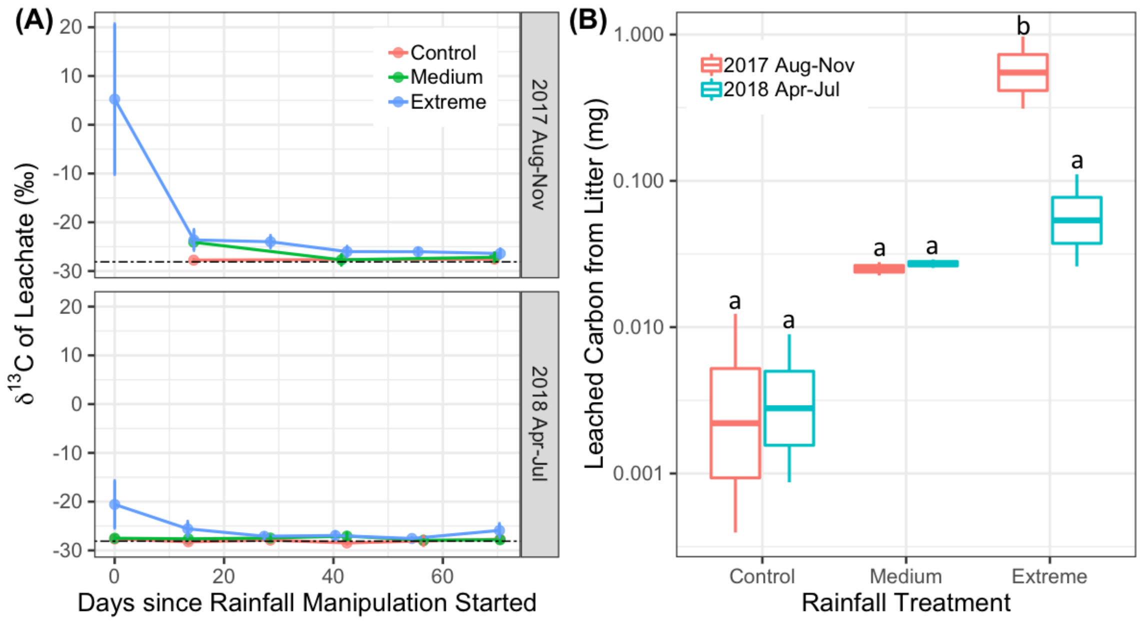

Figure 4-10 (A) Temporal change of δ^{13}C of DOC at 30 cm depth under different rainfall treatments. Error bars represent standard error. Dashed line represents mean of δ^{13}C of DOC from no litter treatments. Statistical tests are in Table 2. (B) Litter-derived carbon as DOC at 30 cm depth under different rainfall treatments. Boxes with different letters are significantly different (Tukey's HSD, $P < 0.05$). Note log scale of y axis.

δ^{13}C of DOC showed different patterns for control, medium, and extreme rainfall treatments (Figure 4-10A). In extreme rainfall treatment, the δ^{13}C of DOC was high after first watering event and then quickly decreased. In control treatment, δ^{13}C was not different from the baseline. δ^{13}C of DOC in the medium rainfall treatment was in between the extreme and control treatments. δ^{13}C of DOC was also higher in 2017 than 2018 (Table 4-1) especially in the beginning under extreme rainfall treatment.

4.3.5 Soil organic carbon (SOC)

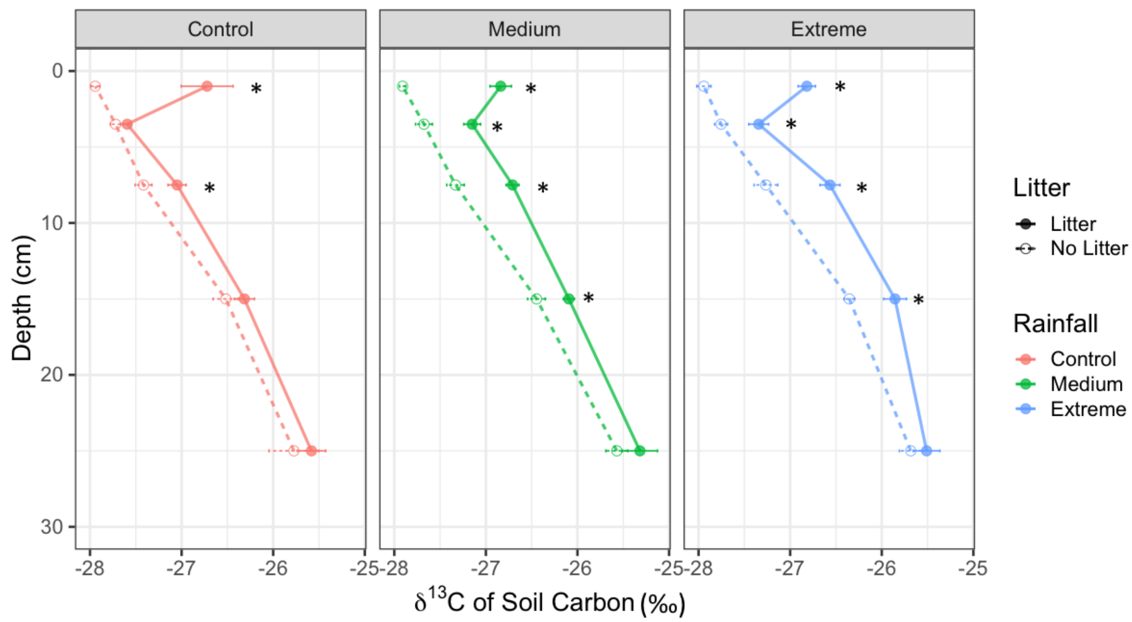

Figure 4-11 δ^{13}C of soil organic carbon at different depths in different treatments. Error bars represent standard error. Stars (*) indicate significant differences between litter and no litter treatments.

With the exception of the surface (0-2 cm) layer, δ^{13}C gradually became less negative with depth (Figure 4-11). δ^{13}C was significantly higher in litter than no litter treatment at 0-2 cm and 5-10 cm depths in all treatments, and at 2-5 cm and 10-20 cm depths in the medium and extreme rainfall treatments. The difference of δ^{13}C was calculated (litter – no litter) if t-test was significant. Either t-test (medium vs. extreme) or ANOVA (all treatments) was performed to test the effect of rainfall on the δ^{13}C difference and no significant effect was found. T-tests were also used to assess the effect of litter on carbon content in different rainfall treatments at different depths and was found to be non-significant (Figure 4-12).

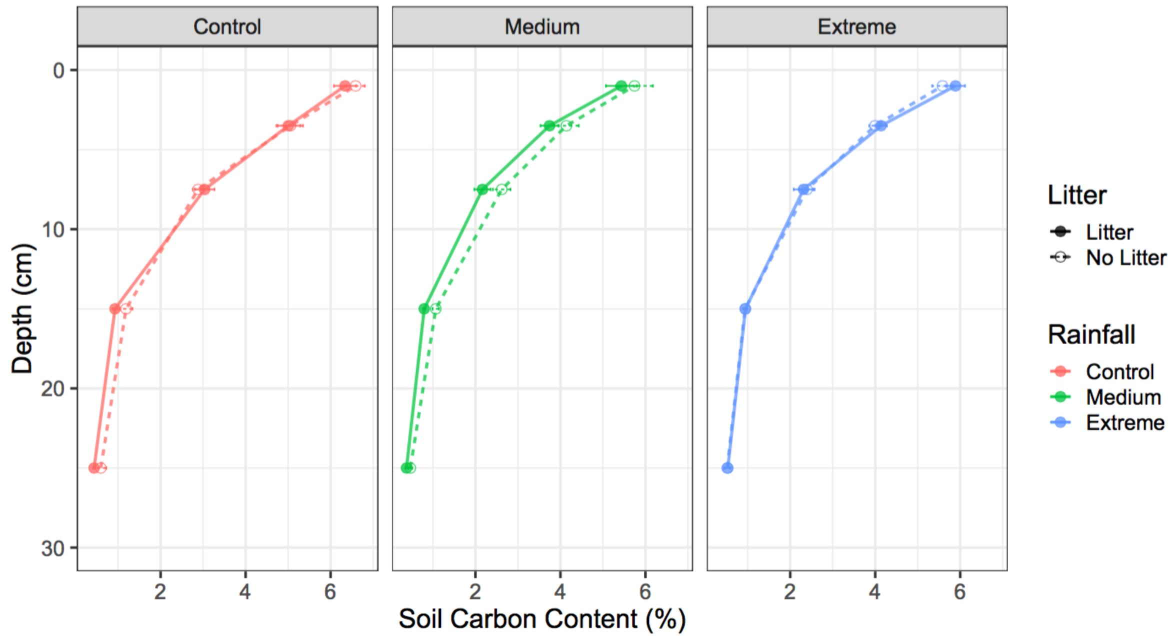

Figure 4-12 Soil carbon content at different depths in different treatments. Error bars represent standard error. No significant difference was found between litter vs. no litter at all depths.

4.4 Discussions

To the best of our knowledge, this is the first *in situ* study to assess the effects of changing rainfall patterns on different pathways of litter carbon during decomposition. The generally higher soil moisture in litter than no litter treatments soils, moisture peaks corresponding to rainfall manipulation events, and the monotonic decrease of moisture between manipulations indicated that our rainfall exclusion was successful most of the time, except when it rained consecutively with over 100 mm rainfall in 5 days in 2018. Year 2018 was one of the wettest years in Maryland history and provided us a unique opportunity to study how soil moisture, which was higher in 2018 than 2017, would affect litter decomposition and soil carbon cycling under different rainfall treatments.

As expected, decomposition rate was lower in extreme than control rainfall treatments, which supports our first hypothesis. While respiration rate was frequently measured, isotopic analysis of CO_2 was not. This and not measuring other potential water pathways such as runoff and lateral water movement prevents full mass balance calculation of litter carbon fates. Still, comparison of relative differences among rainfall treatments, enables us to accept our second hypothesis: under extreme rainfall more litter carbon (DOC and SOC) was transported to deeper soil layers than in control.

4.4.1 Impact of rainfall patterns on litter decay rate

Consistent with our hypothesis, litter decayed faster under control and medium than extreme treatments. First, rainfall affects litter decomposition rate through leaching of soluble compounds (Lensing & Wise, 2007). Leaves under control treatment probably leached more DOC to surface soil due to more frequent rainfall events. Deng et al. (2018) found that an increase of rainfall frequency by 50%, while keeping total amount of precipitation the same, enhanced DOC inputs from litter to soil by 28%. In addition to direct effect of rainfall via leaching, precipitation can also influence decomposition through indirect impacts on the microbes (Salamanca et al., 2003). Infrequent rainfall events could reduce microbial activity due to temporal drought between events (Walter et al., 2013), while regular rainfall events maintains leaf moisture thus more favorable conditions for microbes (Vanlauwe, Vanlangenhove, Merckx, & Vlassak, 1995), resulting in a higher litter decomposition rate.

4.4.2 Litter increased CO_2 efflux only in the beginning of decomposition and extreme rainfall triggered a bigger pulse

Rainfall treatments could induce a pulse of CO_2 efflux from soil due to CO_2 displacement by water in the soil (Huxman et al., 2004) as well as enhanced microbial metabolism (W. Borken et al., 2003). After watering, the accumulated substrates during soil drying became available and increased mineralization rate by microbes (Kim, Vargas, Bond-Lamberty, & Turetsky, 2012). Microbes in the forest soil are generally C-limited (S. Xu, Liu, & Sayer, 2013) and the presence of fresh litter combined with rainfall treatment increased labile carbon input to the soil and increased soil microbial biomass and activity (Fisk & Fahey, 2001), resulting in a higher difference (litter – no litter) of CO_2 efflux, especially right after rainfall events (Figure 4-6). Litter then slowly dried out and contributed less to CO_2 efflux (Figure 4-7) (Lee et al., 2004) on the short term. Over time, as labile carbon is used up, recalcitrant litter components become relatively enriched in the later phase of decomposition (Bjorn Berg, 2000; Couteaux et al., 1995), contributing little to CO_2 efflux. In the later stage, the decreasing contribution of litter to CO_2, combined with litter as a physical barrier on CO_2 movement, resulted in a smaller CO_2 flux in litter than no litter treatment soils. The difference was more negative in 2018 than 2017 because there was more biological activity during the summer of 2018 than in the late fall of 2017, leading to more of the CO_2 efflux being blocked in 2018.

We also observed a higher $\delta^{13}C$ of CO_2 efflux in extreme than control rainfall treatments right after rainfall events, because in extreme events more water with a higher intensity was added to leaves, which also experienced a longer period of drying. The size of the pulse is positively related to amount of water applied (W. Borken et al., 2003; Werner Borken & Matzner, 2009), rainfall intensity (X. Xu & Luo, 2012), and dry periods (L. Yan, Chen, Xia, & Luo, 2014).

4.4.3 Rainfall intensity, initial soil moisture, and timing of heavy rainfall events influence litter carbon transport

Contribution of carbon from leaf litter to leachate DOC was small, even for the extreme rainfall treatment which had the most litter derived carbon, indicating strong retention of DOC by soil. The retention of litter derived DOC is driven by microbial decomposition and physical-chemical adsorption (Klaus Kaiser & Kalbitz, 2012). DOC retention can occur rapidly: K. Kaiser and Zech (1998) reported over two thirds of the added DOC was retained by subsoil horizons within 15 minutes of addition to the soil. Despite high and fast sorption of DOC by soil minerals, we still observed $\delta^{13}C$ signatures of DOC at 30 cm depth in the litter treatments and more litter derived carbon was collected in extreme than both control and medium treatments, especially in 2017. This is in line with a previous study showing direct transfer of organic solutes from the forest floor to subsoil and further to ground water during heavy storm events water (Klaus Kaiser & Guggenberger, 2005). Fast water movement induced by extreme rainfall events can decrease sorption and microbial processing of DOC, causing litter derived DOC to be transported deeper than expected (M. Fröberg et al., 2007; F. Hagedorn et al., 2000). The leached carbon can be incorporated by microbes and interact with soil minerals to form stable SOC (M. Francesca Cotrufo et al., 2015; M. F. Cotrufo et al., 2013). This can explain the longer translocation of litter carbon to deeper soil to form SOC in medium and extreme than control treatments, which supports our second hypothesis.

Despite higher volume of leachate in 2018 than 2017, more litter derived DOC was captured at depth in the latter under extreme treatment. Apart from rainfall intensity, the velocity of water movement in soil is also determined by initial soil moisture. Infiltration rate, which is an indicator of the velocity with which water moves through soil, is affected by initial soil moisture, and decreases with increasing soil water content (Castillo, Gomez-Plaza, & Martinez-Mena, 2003; H. Liu et al., 2011). More rainfall in 2018 kept soil moisture higher, leaving more time for microbes and minerals to retain labile litter carbon due to slower water movement, and eventually led to less litter derived DOC captured at depth.

The high $\delta^{13}C$ value in the extreme treatment was detected only after the first rainfall event. Similar to the CO_2 pathway, and as concentrations of labile, water-soluble carbon decreased (Bjorn Berg, 2000; Couteaux et al., 1995), so did the $\delta^{13}C$ signature in DOC. This points to the importance of the timing of heavy rainfall events on DOC transport. In seasonal forests a heavy rainfall right after leaf fall would transport more litter carbon to deeper horizon than a similar event in summer when decomposition is at a later stage.

Although we detected $\delta^{13}C$ signatures in SOC, the small total amount of litter derived carbon did not change soil carbon content. Our experiment was too short to expect significant changes in subsoil carbon content (Beier et al., 2012). Alternatively, the increased labile carbon input by litter, especially at the beginning of decomposition, could accelerate decomposition of soil organic matter, causing a 'priming effect' (Yakov Kuzyakov, 2010), which could even decrease soil carbon content (Heath et al., 2005). In a laboratory experiment with similar setup to this field experiment, Yang et al. (submitted) detected priming effect. Either way, long-term field manipulation experiments are needed to determine soil carbon changes in either direction as a result of changing precipitation patterns.

4.5 Conclusions

The present study is the first to explore the increase of extreme rainfall events on partitioning of litter carbon during decomposition in the field and results are consistent with a similar experiment conducted in the lab (Yang et al., submitted). Increase of extreme rainfall events would slow down litter decomposition rate and transport more litter derived DOC into deeper horizons, where it may be incorporated by microbes and interact with soil minerals to form SOC. Apart from rainfall intensity, initial soil moisture and timing of extreme rainfall events can influence litter carbon transport. These findings provide new insights into the effect of altered hydrologic cycle on partitioning of litter carbon during decomposition. If the observed phenomena generally apply to temperate forests, the enhanced litter carbon transport during heavy rainfall events would significantly influence soil carbon cycling and global climate system. Some representation of these processes should be included into ecosystem models to better predict how terrestrial carbon stocks respond to changing climate scenarios, especially the increase of extreme rainfall events.

Acknowledgements

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5. Extreme rainfall and leaf litter effects on carbon fluxes in simulated forest soil

Abstract

Forest soils are an important biological sink of methane (CH_4) and source of carbon dioxide (CO_2). Both rainfall patterns and leaf litter inputs are predicted to change in the future, and their effects on CH_4 uptake and CO_2 production in temperate forest soils are not well understood. Here we report soil CH_4 uptake and CO_2 production rates in a six-month laboratory experiment with different rainfall and litter treatments using soils collected from a temperate deciduous forest in Eastern North America. Extreme rainfall triggered a bigger pulse of CO_2 production than control, but rainfall treatments had no effect on cumulative CO_2. Methane uptake rates did not differ between control and extreme rainfall treatments due to high soil moisture conditions. Both CH_4 uptake and CO_2 production rates in litter treatment were higher than those in no litter treatment soils. The change of litter cover needs to be taken into consideration when modeling annual CH_4 and CO_2 fluxes.

5.1 Introduction

Precipitation patterns, including annual amounts, timing, variability and extremity are expected to change in the future all over the world (Beier et al., 2012). Due to a warmer climate, the global water cycle will be intensified: increased duration and intensity of drought and storms, whether thunderstorms or snow blizzard, is expected to occur even in places where total precipitation is decreasing (Trenberth, 2011). In the Northeastern United States, most model simulations project an increase in the frequency of intense rainfall events despite no change in total summer precipitation (Hayhoe et al., 2006). Rainfall is a major driver of soil water content (SWC), which strongly influences belowground biogeochemical processes, including CO_2, CH_4 production and transport, and CH_4 consumption.

Both CO_2 and CH_4 are important greenhouse gases and their increase in the atmosphere since pre-industrial times is the main cause of global warming (IPCC, 2013; Megonigal & Guenther, 2008; Pitz et al., 2018). Soil respiration, including both respiration from root and soil biota and decomposition of litter and soil organic matter by microbes, represents the largest carbon flux from land to atmosphere (B. Bond-Lamberty & Thomson, 2010a; Ben Bond-Lamberty et al., 2004). At a rate of 68 – 80 Pg C yr^{-1} (Raich, 1995; Raich & Schlesinger, 1992), soil respiration is almost an order of magnitude larger than anthropogenic fossil fuel combustion, which is estimated as 9.4 ± 0.5 Pg C yr^{-1} (Le Quere et al., 2018).

In addition to CO_2 production in upland soils, CH_4 uptake is a small but important flux in the global budget, accounting for 30 Tg $year^{-1}$ or about 6% of the global sink (Kirschke et al., 2013; Knief et al., 2003; Saunois et al., 2016), with forests representing approximately 50% of this sink (Dutaur & Verchot, 2007; Fest et al., 2017). Forest soils produce and consume CH_4 (von Fischer & Hedin, 2002). Megonigal and Guenther (2008) suggest that upland soils harbor populations of methanogens and are capable of becoming net sources of CH_4 when sufficiently wet to create anoxic conditions. In temperate forest soils, oxidation generally exceeds production and results in a net uptake of CH_4 from atmosphere (Dutaur & Verchot, 2007; Nazaries et al., 2013; Ullah & Moore, 2011).

Net fluxes at the soil-atmosphere interface depend on biological activity and gas diffusivity, both of which are affected by soil water content (SWC). The relationship between

SWC and gas fluxes is usually described by a parabolic or 'hump-shaped' curve. At very low SWC, biological activity is limited, resulting in low CH_4 (W. Borken & Beese, 2006; Bowden, Newkirk, & Rullo, 1998) and CO_2 flux (Moyano et al., 2013; Schimel et al., 2007) due to osmotic stress and low substrate diffusion. At high SWC, low diffusivity of CH_4 and oxygen in water compared to air (Marrero & Mason, 1972) limits both CH_4 oxidation and CO_2 production.

The leaf litter layer plays multiple roles on the forest floor. In addition to being a major resource for decomposers, the litter layer regulates soil microclimatic conditions by forming a protective layer on the soil surface (Sayer, 2006; S. Xu et al., 2013), and can act as a physical barrier against gas exchange. Field studies (Dong et al., 1998; Leitner et al., 2016; Wang et al., 2013) have demonstrated that leaf litter addition can reduce CH_4 uptake in some soils. A meta-analysis of *in situ* litter manipulation experiments showed that soil respiration rates increased with litter addition and decreased with litter removal (S. Xu et al., 2013).

Both the quantity and quality of litter inputs are expected to change in the future. Global and regional changes such as elevated CO_2, nitrogen deposition, and temperature increase can enhance plant productivity, thereby increasing litter inputs (McMahon, Parker, & Miller, 2010). Altered rainfall patterns could affect tree phenology and tree species distribution (Condit et al., 1996), leading to shifts in both quality and quantity of litter inputs to soil. In addition, extreme events could also result in sudden, sometimes dramatic changes in litter inputs, such as large increase in aboveground litterfall after hurricanes or severe storms (Ostertag et al., 2003).

Most experimental studies of soil CH_4 uptake have only considered water addition or removal (Billings et al., 2000; W. Borken et al., 2000; W. Borken et al., 2006; Kim et al., 2012).

Blankinship et al. (2010) found that CH_4 uptake rates in the cold and wet ecosystems decreased with increasing precipitation, especially during the wet season. Increase of CH_4 uptake by forest soil, especially under dry conditions, has been shown in rainfall reduction studies (W. Borken et al., 2006; Fest et al., 2017). Rewetting of a dry soil can cause a large pulse of CO_2 to be released due to physical replacement of CO_2 with water and enhanced microbial activity (Werner Borken & Matzner, 2009; Huxman et al., 2004; Miller, Schimel, Meixner, Sickman, & Melack, 2005). The size of the wetting pulse increased with the rainfall amount, intensity, and duration of drying (W. Borken et al., 2003; Werner Borken & Matzner, 2009; Miller et al., 2005). Intensity of rainfall had no effect on cumulative CO_2 fluxes when equal amount of water was applied (Hentschel et al., 2007; Muhr, Goldberg, Borken, & Gebauer, 2008). How intensity and frequency of rainfall events interact with leaf litter to influence short and long-term response of CO_2 flux, has been less studied.

We conducted a laboratory experiment to examine the interaction of rainfall intensity and frequency and leaf litter cover on CH_4 and CO_2 fluxes in forest soils. Control and extreme rainfall treatments were determined based on decades of local historical precipitation data. The total amount was kept constant while control treatment had lower intensity but higher frequency rainfall events and extreme treatment had higher intensity but lower frequency rainfall events. We hypothesized that (a) leaf litter would reduce CH_4 uptake and increase CO_2 production, (b) extreme rainfall treatment would trigger a greater CO_2 pulse than control, but cumulative CO_2 would not differ, and (c) CH_4 uptake in the extreme rainfall treatment would exceed that in the control rainfall treatment due to lower soil moisture.

5.2 Method

5.2.1 Research site and soils

This study was conducted using soils from a mature forest stand at the Smithsonian Environmental Research Center (SERC). SERC is located along the west coast of Chesapeake Bay in Edgewater, MD (38º53'N, 76º33'W). The mean annual precipitation in the region is 1,146 mm and the mean annual temperature is 13ºC (Correll et al. unpublished data). The forests in this area are a mosaic of stands differing in age, past land use and tree composition (Pitz et al., 2018; Yesilonis et al., 2016). Soils for the experiment were taken from a 150 year stand dominated by tulip poplar (*Liriodendron tulipifera*), sweet gum (*Liquidambar styraciflua*), oaks (*Quercus spp*.), American beech (*Fagus grandifolia*), and hickories (*Carya spp*.). The soil type is Collington (Typic Hapludult, fine sandy loam) (Yesilonis et al., 2016). Total carbon content at 0-10 and 10-20 cm is 3.04% and 1.05%, respectively, and pH ($CaCl_2$) at 0-10cm and 10-20cm is 4.8 and 4.7, repectively (Yesilonis et al., 2016). There is little persistent forest litter layer and a thin O horizon in this site because of presence of earthworms, both native and non-native (Szlávecz & Csuzdi, 2007; Szlavecz et al., 2011).

5.2.2 Laboratory experiment setup

In laboratory studies focusing on methane uptake and CO_2 production, small mesocosms are typically used, and data are collected for a short amount of time (Blankinship et al., 2010; Bowden et al., 1998; Miller et al., 2005; Nesbit & Breitenbeck, 1992). We planned our rainfall manipulation experiment for six months, which required larger soil volume. We opted for medium size mesocosms, which are often used in soil ecology and biogeochemistry experiments (Crumsey et al., 2015; Setälä et al., 1990). Taking undisturbed soil monoliths of this size from forests is very challenging due to the extensive root biomass. As a compromise between undisturbed and completely homogenized soils, the so-called 'simulated forest floor' approach has been proposed (Huhta & Setälä, 1990), in which soil horizons were recreated and even small scale microhabitat heterogeneities were introduced. We followed the first step on this protocol. Specifically, we collected soils from our study site in September 2016 from the top 10 cm (surface soil) and from the deeper (20-40 cm) mineral layer (subsurface soil). Both soil layers were sieved through a 4 mm sieve and roots and leaves were removed. Soil was reconstructed in transparent acrylic columns, 19.0 cm in diameter. At the bottom, a 10 cm deep clean gravel layer was added to filter leachate and prevent anaerobic conditions. The gravel was covered with 2 mm mesh and topped with 6.0 and 2.8 kg subsurface and surface soil, respectively. This amounted to a 25 cm high soil column (15 and 10 cm), with bulk densities similar to those found in the field at SERC's mature forests (Yesilonis et al., 2016). Each column had an approximate 10 cm headspace. A more detailed description is in Yang et al., (submitted).

Volumetric water content (VWC) at 8 cm depth was monitored by ECH_2O EC-5 moisture sensors (METER Group, Pullman, Washington, USA). Lab temperature and relative humidity were monitored continuously by Maxim's iButton (Maxim Integrated, San Jose, California, USA). Leachates were collected using plastic bottles after rainfall events. At the end of the experiment gravimetric water content (GWC) was determined by drying the soil samples at 105 ºC until constant mass to fully characterize soil moisture conditions at all depths.

5.2.3 Rainfall treatments

Historical precipitation data in SERC to determine control and extreme rainfall treatments

The two rainfall treatments established in the lab were based on historical precipitation data from SERC and surrounding weather stations. Rainfall intensity was derived from 15-minute precipitation data from US Custom House in Baltimore (1984 – 1999) (https://www.ncdc.noaa.gov/cdo-web/datatools). Frequency of heavy rainfall events was derived from daily precipitation data from US Naval Academy (1894 – 1976) (https://www.ncdc.noaa.gov/cdo-web/datatools) in Annapolis, Maryland and SERC (2002 – 2013). A comparison of historical precipitation and manipulated rainfall patterns is in Yang et al., (submitted). Both the frequency and intensity of rainfall were manipulated, while total rainfall delivered during the experiment remained constant. The intensity and frequency of rainfall events in the control treatment were the historical average of 15-minute rainfall intensity and average frequency of rainy days respectively. In extreme treatment, the intensity and frequency were top 1% of 15-minute rainfall intensity and top 1% of frequency of rainy days respectively. As a result, during a four-week period, the extreme treatments received two heavy rainfall events with high intensity, while the control treatments received eight rainfall events with low intensity. A more detailed description is in Yang et al., (submitted).

Rainfall manipulation

Before we added leaf litter to the soil columns, soil moisture was raised to field capacity by gradually adding deionized water over the course of 20 days. Upon reaching field capacity, 7.0 g (dry mass) of tulip poplar *(L. tulipifera)* leaf litter was placed on the soil surface in litter treatment columns. To ensure relatively homogeneous litter quality, long petioles were removed, and leaves were broken up by hand and sieved through an 8-mm sieve. The 7.0 g of leaf litter added to each soil column equaled 233g/m^2, similar to the tulip poplar litter fall input in old forest stands at SERC (Szlavecz et al., 2018).

There were two rainfall manipulations, control and extreme, and two leaf litter manipulations (detailed below), with each combination replicating three times. The four treatments hereafter are labeled as CL (control-litter), CNL (control-no litter), EL (extreme-litter), and ENL (extreme-no litter). In the control rainfall treatment, 300 ml deionized water was added to column in 2.5 hours. In extreme treatment, 1200 ml deionized water was added to column in 1.5 hours. In both treatments, water was added by a 50 ml syringe gradually in 15-minute intervals to be evenly distributed. When soil moisture reached 35% we stopped watering for one week or reduced the amount to avoid complete saturation and anoxic conditions (Yang et al., submitted). Rainfall manipulations started in March and ended in September 2017.

5.2.4 CH_4 and CO_2 flux measurements

Static chamber method was used to determine CO_2 and CH_4 fluxes. A PVC lid assembled with a CO_2 sensor (GMP 343, Vaisala, Vantaa, Finland) was placed on the experimental columns. CO_2 concentrations in the headspace were recorded every second for 6 minutes. CO_2 flux was measured mostly daily, with more frequent measurements after rainfall events. When taking CH_4 flux measurement, an airtight lid with septa was placed on top of the plastic column. A total of three or four gas samples of 60 ml were collected at different CH_4 concentrations using

Cali-5-Bond air & gas sampling bags (Calibrated Instruments Inc., McHenry, Maryland, USA). Methane concentration was determined by a cavity ring-down spectroscopic carbon isotope analyzer (Picarro G2101-i, Picarro Inc., Santa Clara, California, USA) connected to an automated sampling manifold (Picarro A0311). We occasionally dropped a concentration data point because of poor quality data from the analyzer. If a concentration data point was dropped, both CH_4 and CO_2 data had to support that decision. In every case, the slope was based on ≥ 3 observations. For a slope to be determined as a quality data point, the R^2 had to be greater than 0.80. Gas flux rate was calculated as:

$$F = \frac{dC}{dT} \times \frac{V}{S}$$

where F is the gas flux in μmol m^{-2} s^{-1}, C is the mole concentration in μmol m^{-3}, T is time, V is the volume of headspace, and S is the area of the soil surface in the chamber. dC/dT can be approximated as slopes of fitted lines between CO_2 or CH_4 concentrations and time.

Methane flux rates were measured monthly from March to August 2017 for a total of five campaigns. When CH_4 samples were taken, CO_2 efflux rates were also measured within half an hour. To gain insight on how CH_4 flux would change after single rainfall event, four time points were selected for gas sampling in both control and extreme rainfall treatments. In general, CH_4 flux rates were measured within 30 minutes (T_{C1}), 4 hours (T_{C2}), 1 day (T_{C3}), and 3.5 days (T_{C4}) after control columns received a rainfall treatment. While for extreme rainfall treatment, CH_4 flux rates were measured within 30 minutes (T_{E1}), 2 days (T_{E2}), 1 week (T_{E3}), and 2 weeks (T_{E4}) after rainfall event. An illustration of the sampling scheme is in Yang et al., (submitted). In March, CH_4 uptake rates were only measured in one column in each treatment.

In April, two columns were measured in each treatment. For the following three campaigns of measurements, all three columns in each treatment were measured, and litter treatments columns were measured at 4 time points, while CNL treatment columns were only measured at T_{C4} after rainfall treatment and ENL treatment columns were only measured at T_{E3} after rainfall treatment.

5.2.5 Statistical analysis

All statistical analyses were conducted using R version 3.3.3 (R Core Team, 2017). P values below 0.05 were considered significant and those between 0.05 and 0.1 were considered nearly significant. Two-way analysis of variance (ANOVA) followed by the Tukey's HSD for multiple comparisons was used to assess the effect of rainfall and litter treatments on cumulative CO_2 flux. Mixed effect models were conducted using the *lme4* package (Bates et al., 2015) to evaluate litter and rainfall effect on GWC, CH_4 and CO_2 flux rates. For GWC, column number was treated as random effect, and depth was treated as one fixed effect. As we later found significant interactions between litter and rainfall, we conducted separate analyses for rainfall and litter. To evaluate the effect of rainfall on GWC, different models were run for litter and no litter treatments separately. To evaluate the effect of litter on GWC, different models were run for control and extreme rainfall treatments separately. Factors were evaluated in the models following the column number, depth, rainfall or litter order. For CH_4 and CO_2 flux rates, column number was treated as random effect. To evaluate the effect of litter on CH_4 and CO_2 flux rates, separate analysis was conducted for control and extreme rainfall treatment using rates at T_{C4} and at T_{E3}, respectively. Sampling weeks, and litter were treated as fixed effects. Factors were evaluated in the models following the above order. To evaluate the effect of rainfall on CH_4 and CO_2 flux rates, only data from the litter treatment were used due to limited sampling in no litter treatment. Methane and CO_2 flux rates were compared separately between T_{C1} and T_{E1} (right after water addition), and between T_{C3} and T_{E2} (1 day and 2 days after water addition respectively). Sampling weeks, and rainfall were treated as fixed effects. Factors were evaluated in the models in the above order. Mixed effect models were also conducted on the transient response of CO_2 flux before and after rainfall treatments in similar time frame. The first two weeks of rainfall treatments were excluded because the experiment had not reached a steady state. Column number was treated as random effect, and sampling weeks, watering, litter, rainfall and their interaction were treated as fixed effects. For all mixed models, likelihood ratio tests were used to assess significant differences between nested models, and were followed by the Tukey's HSD test for multiple comparisons using *multicomp* and *emmeans* packages. Linear regression was used to evaluate the correlation between CH_4 flux rates, CO_2 efflux rates, and temperature, VWC, and time since start of experiment (in days).

5.3 Results

At the end of the experiment, leaf litter mass loss was 67.3 ± 3.22% and 60.8 ± 3.88% (mean ± SE) in the control and extreme rainfall treatments, respectively. Total volume of leachate (mean ± SE) was 242.8 ± 64.4 ml, 1151.7 ± 44.3 ml and 866.4 ± 172.1 ml for ENL, EL and CL treatments, respectively. No leachate was collected in CNL treatment. Room temperature was monitored continuously and stayed above 20 ºC most of the time (Figure 5-1). Mean temperature during the experiment was 21.9 ºC.

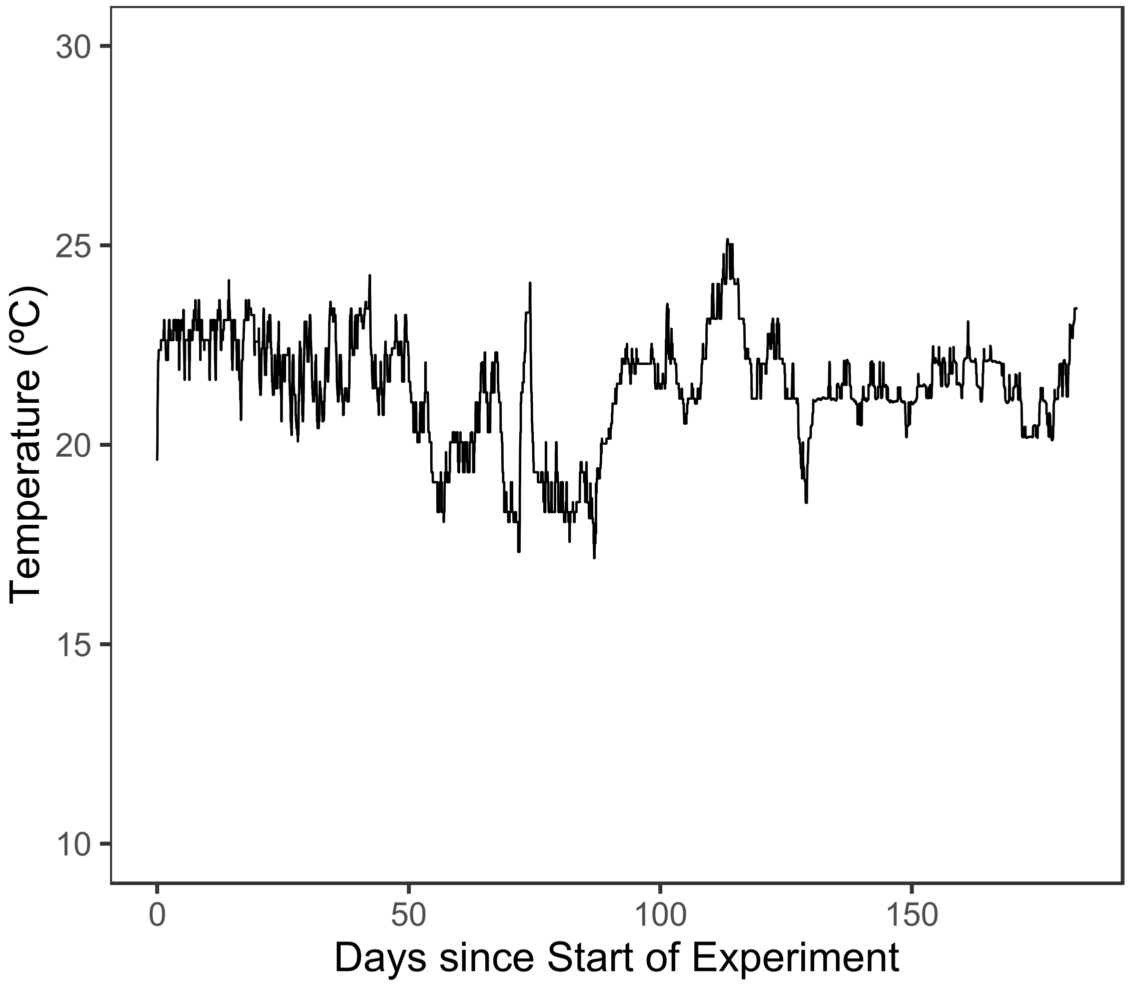

Figure 5-1 Air temperature in the laboratory during the experiment

5.3.1 Water mass balance and soil moisture

During the experiment, evaporation contributed to the most water loss and was higher in litter than no litter treatment soils (Table 5-1, Table 5-2). Litter treatment soils had a higher volume of leachate than no litter treatment soils. Litter × rainfall interaction effect was nearly significant in soil water pathway; a detailed comparison of GWC between different treatments at different depths is discussed later.

Table 5-1 Partitioning of water in different treatments. Numbers are percentages of total amount (8700 mL) of rainfall added to soil columns throughout the experiment. The water was collected at the bottom as leachate, stored in the soil, or lost to the atmosphere (N = 3).

		Evaporation	Soil moisture	Leaching
Control	Litter	77.8	9.2	13.0
	No Litter	88.8	9.6	1.6
Extreme	Litter	76.0	8.3	15.7
	No Litter	91.5	4.4	4.1

Table 5-2 P values for Two-way ANOVA evaluating litter and rainfall effects on different pathways of water

	Litter	Rainfall	Litter×Rainfall
Evaporation	< 0.001	0.844	0.333
Soil	0.113	0.015	0.062
Leaching	< 0.001	0.127	0.963

Volumetric water content (VWC) (%) at 8 cm depth was measured throughout the study (Fig. 1). VWC ranged from 11.2 to 48.0, from 9.9 to 47.8, from 10.2 to 50.3, and from 9.3 to 47.6 in CNL, CL, ENL and EL treatments, respectively. The mean VWC was 35.4 ± 0.03 (CNL), 38.1 ± 0.02 (CL), 35.2 ± 0.04 (ENL), and 38.3 ± 0.02 (EL). Each abrupt increase of VWC (Figure 5-2) corresponded to a rainfall event. Litter treatment soils always had a higher VWC than no litter treatment soils in extreme rainfall treatment throughout the experiment (Figure 5-2). While in control rainfall treatment, this was the case only initially: the difference between litter and no litter treatment decreased over time and after week 19 the trend reversed (Figure 5-2).

Gravimetric water content (GWC), measured only at the end of the experiment, confirmed this pattern throughout the entire soil column (Figure 5-3). In all four treatments GWC varied significantly with depth, leaf litter had a significant effect only in the extreme rainfall treatment, and the effect of rainfall was stronger in no litter soils, than in soils with litter cover (Table 5-3, Figure 5-3).

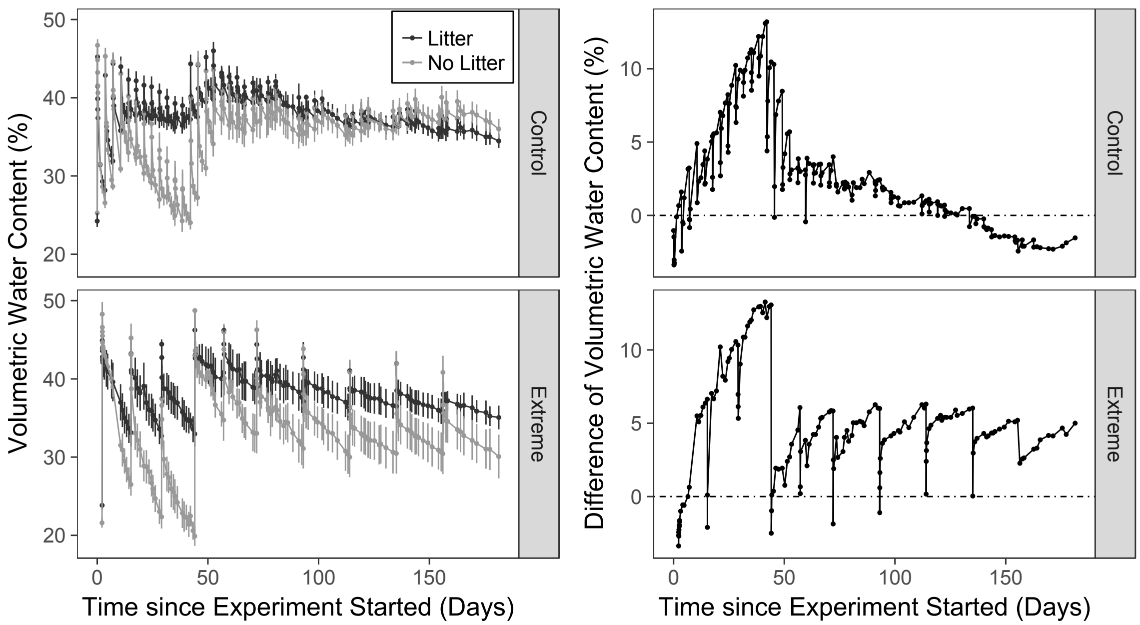

Figure 5-2 Mean (± SE) soil VWC at 8 cm depth (A: n = 3 for each point), and difference of mean VWC between litter and no litter treatments (B: $VWC_{litter} – VWC_{no\ litter}$). Dashed horizontal line: no difference

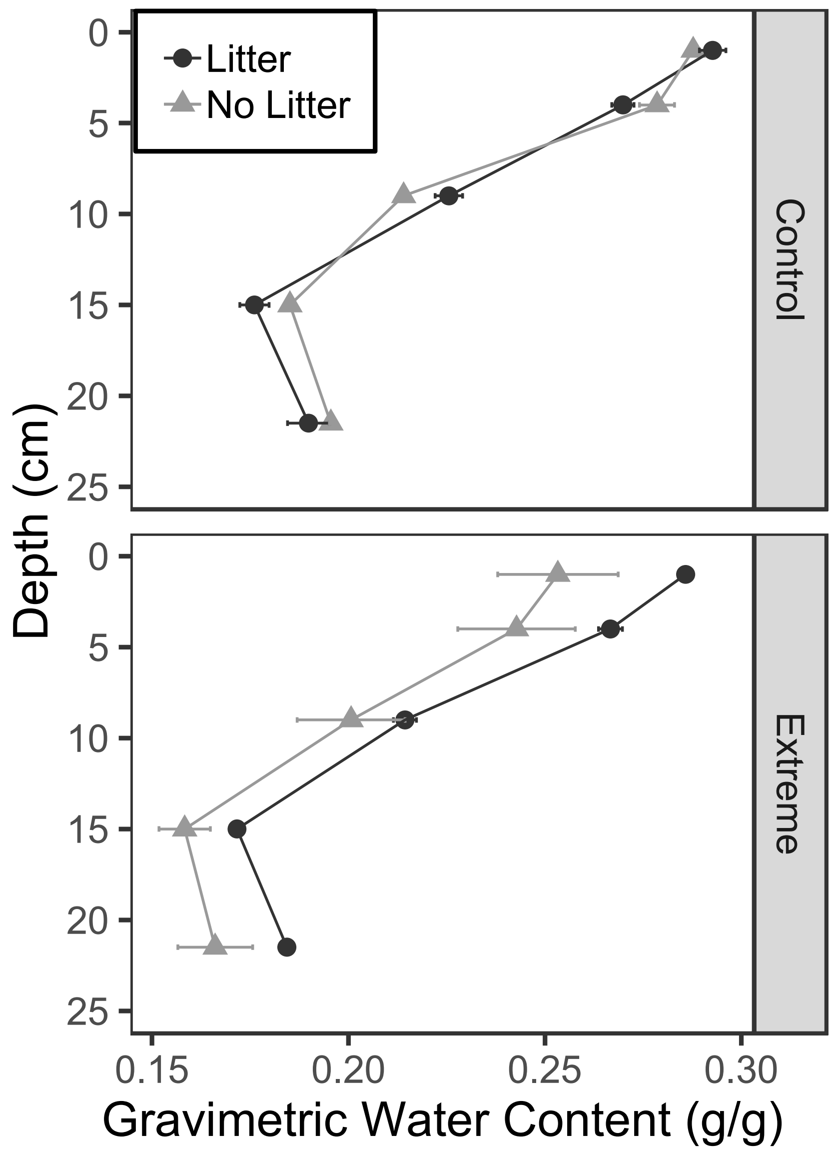

Figure 5-3 Gravimetric water content at the end of experiment at different depths. Circles: litter treatment; triangles: no litter treatment. Error bars represent standard error (n=3).

Table 5-3 Results of mixed effect models testing the effects of litter and rainfall treatments on gravimetric water content (GWC, g/g)

		Depth		Litter		Rainfall	
		χ^2	P	χ^2	P	χ^2	P
Litter effect	Control	119.78	< 0.001	ns	ns	–	–
	Extreme	64.15	<0.001	4.34	0.037	–	–
Rainfall effect	Litter	141.9	< 0.001	–	–	3.63	0.057
	No litter	64.13	<0.001	–	–	5.62	0.018

ns: not significant; –: variable not applicable for analysis

5.3.2 Methane flux

Table 5-4 Results of mixed models testing the effect of litter, and rainfall treatments on CH_4 and CO_2 flux. (T: time point after water addition; subscript C: Control; subscript E: Extreme; subscript numbers: the order of time points after watering. See methods for more details.)

		Weeks		Litter		Rainfall	
		χ^2	P	χ^2	P	χ^2	P
Litter effect	CH_4 rate at T_{C4}	15.95	0.003	14.64	<0.001	–	–
	CH_4 rate at T_{E3}	40.37	<0.001	14.93	<0.001	–	–
	CO_2 rate at T_{C4}	9.87	0.042	19.04	<0.001	–	–
	CO_2 rate at T_{E3}	40.37	<0.001	14.93	<0.001	–	–
Rainfall effect	CH_4 rate T_{C1} vs T_{E1}	22.09	<0.001	–	–	ns	ns
	CH_4 rate T_{C4} vs T_{E2}	15.49	0.004	–	–	ns	ns
	CO_2 rate T_{C1} vs T_{E1}	18.49	<0.001	–	–	8.53	0.004
	CO_2 rate T_{C4} vs T_{E2}	15.93	0.003	–	–	12.16	<0.001

ns: not significant; –: variable not applicable for analysis

A total of 108 flux measurements were made during the study: 38 measurements in EL treatment, 39 in CL treatment, 19 in ENL treatment, and 12 in CNL treatment. In all but one occasion, soil was a CH_4 sink. Soil CH_4 fluxes ranged from -9.78 to -1.30 μg m^{-2} h^{-1} in CL treatment, from -5.79 to -0.51 μg m^{-2} h^{-1} in CNL treatment, from -12.7 to 1.82 μg m^{-2} h^{-1} in EL treatment, and from -8.18 to -0.13 μg m^{-2} h^{-1} in ENL treatment. In all treatments CH_4 uptake decreased as the experiment proceeded (Figure 5-4, Table 5-4). Combining all CH_4 measurements by treatment yielded mean rates of -5.27 ± 1.28 μg m^{-2} h^{-1} (CL), -2.07 ± 1.40 μg m^{-2} h^{-1} (CNL), -5.31 ± 1.57 μg m^{-2} h^{-1} (EL), and – 2.61 ± 1.48 μg m^{-2} h^{-1} (ENL). Methane fluxes were higher in litter treatment than no litter treatment soils for both control and extreme rainfall treatments (Figure 5-4, Table 5-4). Methane fluxes were not significantly different in different rainfall treatments (Table 5-4).

Soil CH_4 uptake rates were positively correlated with soil CO_2 efflux (Figure 5-7, Table 5-5). Methane flux rates were negatively correlated with VWC only in CNL, and had a weak positive significant correlation with VWC in CL (Table 5-5). Temperature effect on CH_4 flux rates was not significant (Table 5-5).

Table 5-5 Results of linear regression models testing the effects of soil moisture, temperature, CO_2 efflux, and time since beginning (TSB) on CH_4 uptake and CO_2 efflux. CL: Control-Litter, CNL: Control-No litter, EL: Extreme-Litter, ENL: Extreme-No litter

		TSB		Temperature		CO_2 efflux		Soil VWC	
		R^2	P	R^2	P	R^2	P	R^2	P
CH_4	CL	0.23↓	0.002	ns	ns	0.09↑	0.060	0.10↑	0.052
	CNL	0.61↓	0.003	ns	ns	0.45↑	0.018	0.57↓	0.005
	EL	0.74↓	< 0.001	ns	ns	0.15↑	0.018	ns	ns
	ENL	0.70↓	< 0.001	ns	ns	0.68↑	< 0.001	ns	ns
CO_2	CL	0.19↓	0.006	ns	ns	–	–	0.32↑	< 0.001
	CNL	0.37↓	0.037	0.26↑	0.089	–	–	0.50↓	0.010
	EL	0.13↓	0.024	0.18↑	0.007	–	–	0.11↑	0.039
	ENL	0.50↓	< 0.001	0.22↑	0.044	–	–	ns	ns

VWC: volumetric water content
↓: negative effect; ↑: positive effect; ns: not significant; –: variable not applicable for analysis

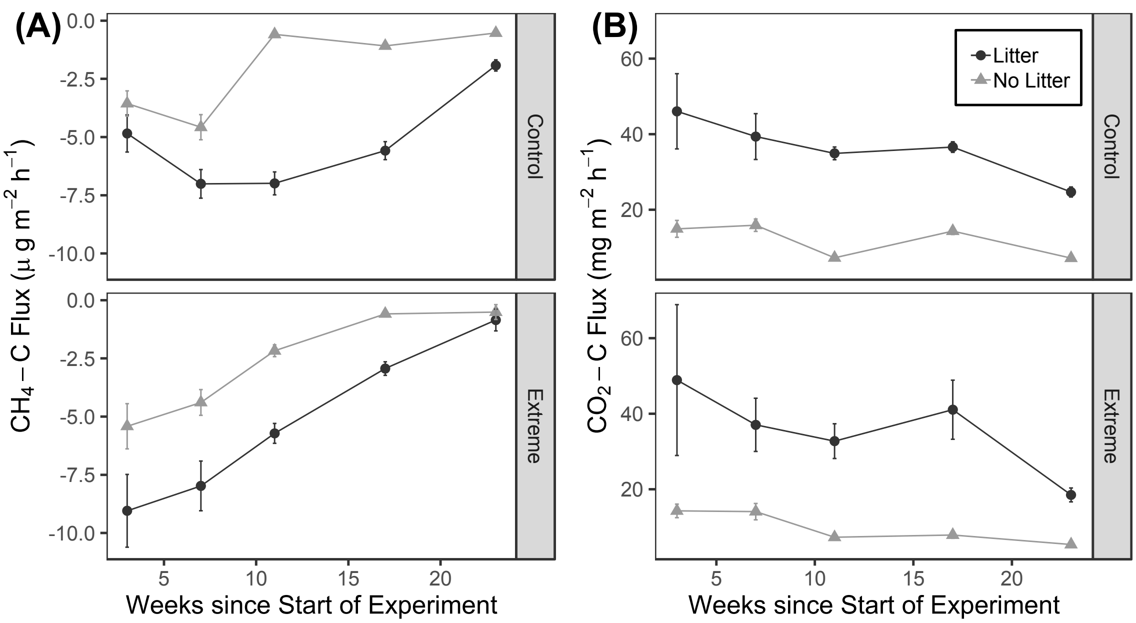

Figure 5-4 Temporal change of CH_4 (A) and CO_2 (B) flux in different rainfall and litter treatments. Circles: litter; triangles: no litter. Error bars represent standard error. Note the different units on y axis

5.3.3 CO_2 flux

CO_2 flux was measured more frequently (a total of 3663 data points were collected throughout the experiment), allowing us to explore the soil system responses to different precipitation events. Litter treatment soils always had a higher CO_2 flux than no litter treatment, regardless of rainfall intensity and timing, i.e. whether the measurement was made before or after watering ($p < 0.001$, Figure 5-5, Table 5-6). Watering triggered a pulse of CO_2 flux in CL ($p < 0.001$), EL ($p < 0.001$), and ENL ($p = 0.020$) treatment, but not in CNL treatment (Fig. 3). The pulse was higher in EL than CL treatment ($p < 0.001$) and ENL treatment soils had a higher CO_2 flux than CNL after water addition ($p < 0.001$) (Figure 5-5). The pulse of CO_2 flux was positively correlated with the change of VWC in EL ($R^2 = 0.29$, $p = 0.004$) and CL ($R^2 = 0.69$, $p < 0.001$) treatment, but negatively correlated in ENL treatment ($R^2 = 0.21$, $p = 0.016$) (Figure 5-6).

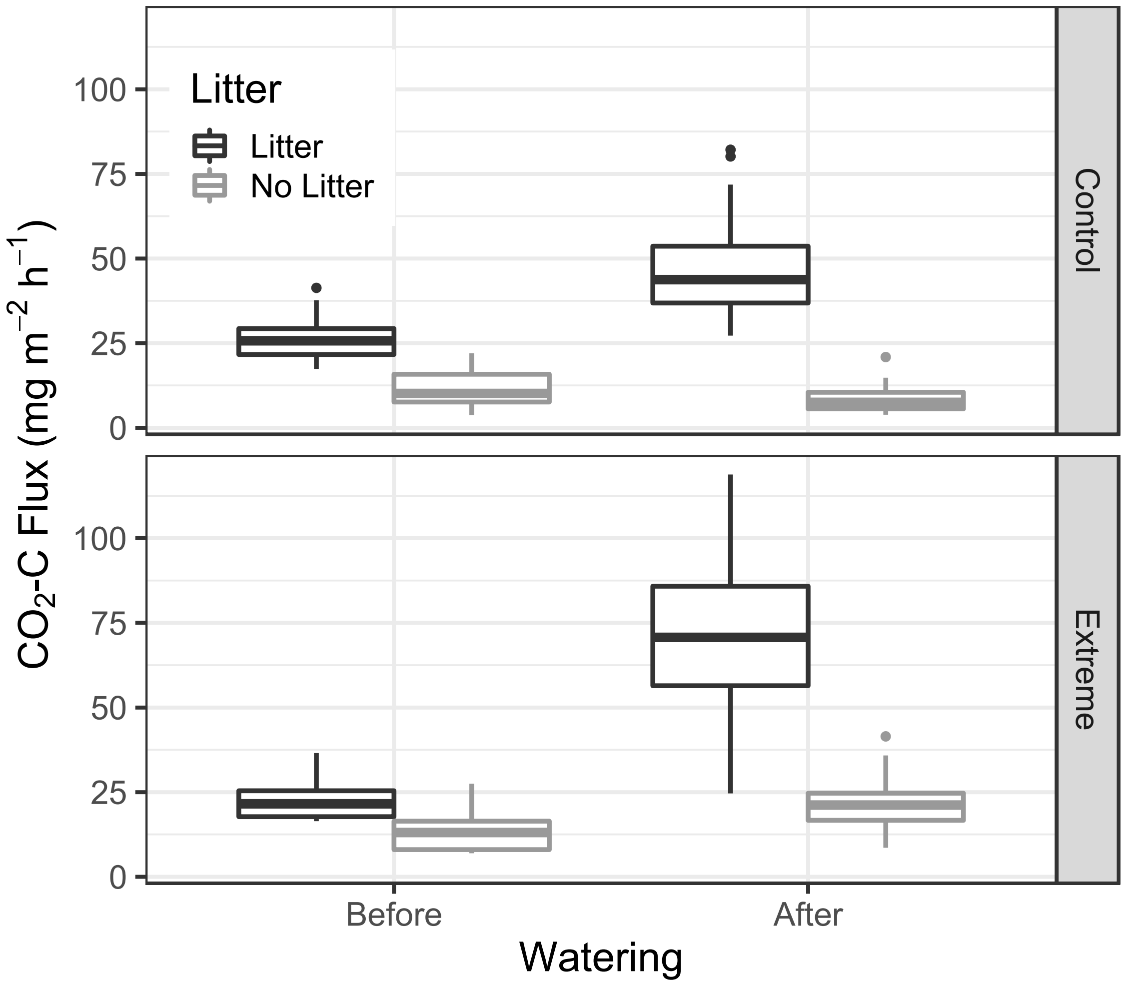

Figure 5-5 CO_2 flux before and after water addition in different treatments. Data for the duration of the experiment were pooled except the first two weeks.

Table 5-6 Results of mixed models testing effect of watering, rainfall, and litter treatment on CO_2 flux

	Weeks		Watering		Litter		Rainfall		Litter×Rainfall×Watering	
	χ^2	P	χ^2	P	χ^2	P	χ^2	P	χ^2	P
CO_2	30.9	<0.001	86.6	<0.001	28.1	<0.001	17.5	<0.001	165.4	< 0.001

We evaluated the short-term response of the systems right after rainfall. CO_2 flux was higher in extreme than control rainfall treatment right after and around 1-2 days after water addition in litter treatment soils (Table 5-4, Figure 5-8). Cumulative CO_2-C was 3927.1 ± 189.6

mg, 3599.3 ± 176.0 mg, 1330.8 ± 59.6 mg, and 1513.3 ± 35.1 mg for CL, EL, CNL, and ENL treatment respectively (Figure 5-9). Only litter was found to be significant on cumulative CO_2 flux ($p < 0.001$). CO_2 showed a decreasing trend throughout the experiment. Soil CO_2 flux rates were positively correlated with temperature in CNL, EL, and ENL treatments, positively correlated with VWC in CL and EL treatments, and negatively correlated with VWC in CNL treatment (Table 5-5).

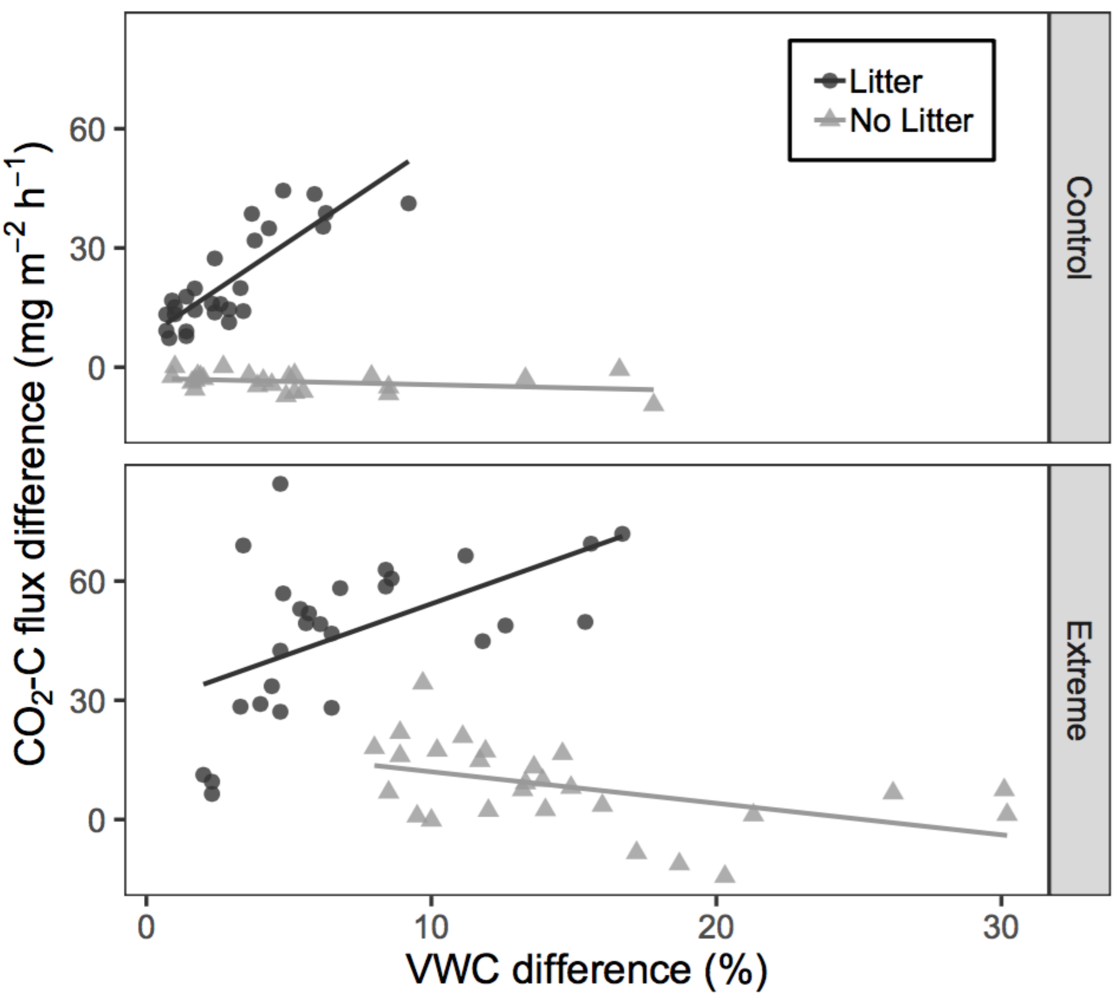

Figure 5-6 Relationship between differences of CO_2 flux versus differences of volumetric water content (VWC) after rainfall treatments. The differences were calculated as values after rainfall minus values before rainfall (in absolute amount) for both CO_2 and VWC.

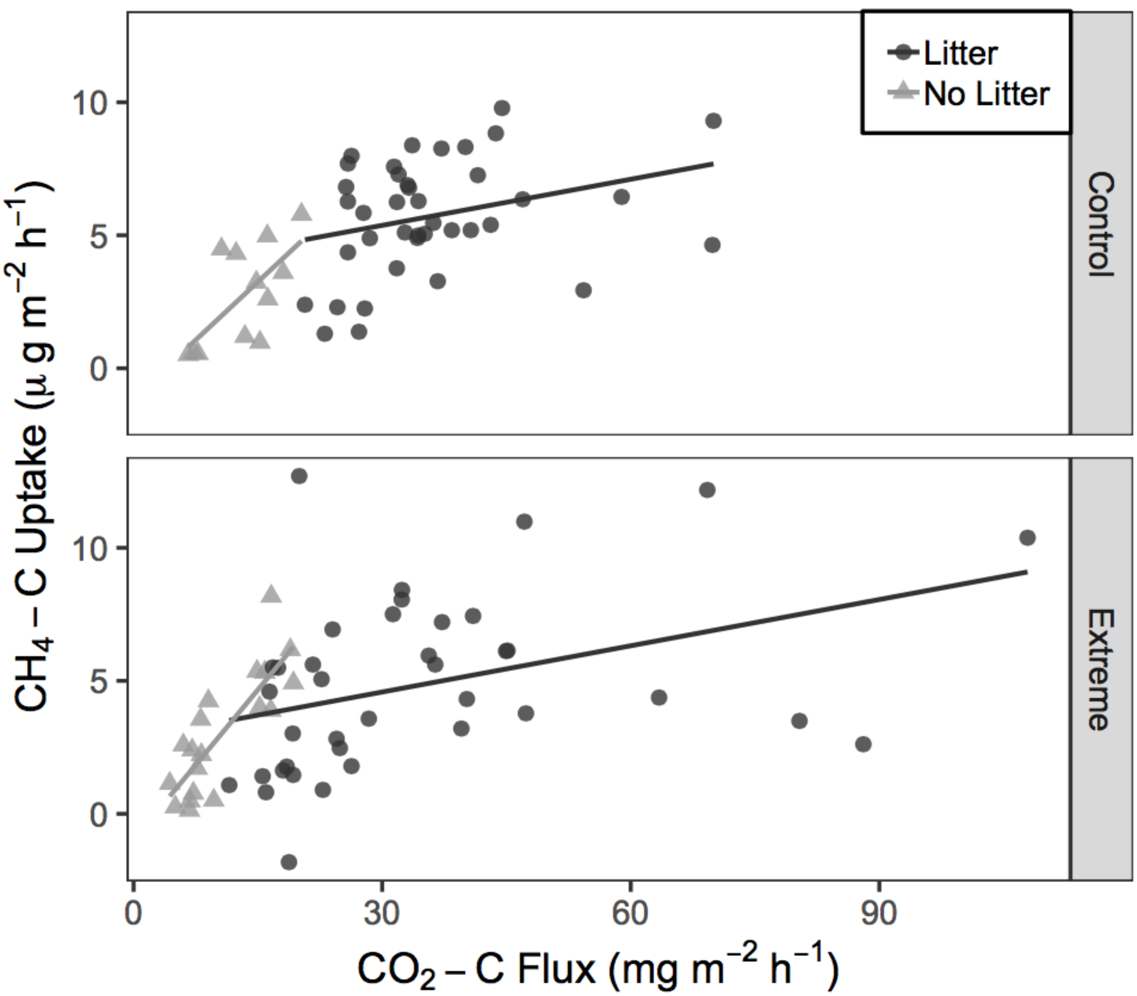

Figure 5-7 Correlation between CH_4 uptake rate and CO_2 efflux rate in various treatments. Circles: litter treatment; triangles: no litter treatment. R^2 and P values are in table 5-5. Note the different scales and units on x and y axes.

5.4 Discussion

Leaf litter forms an interface between the soil surface and atmosphere thus regulating water and gas exchange (Sayer, 2006). Under optimal moisture conditions, the litter layer acts as a barrier against gas exchange at the soil-atmosphere interface; removing leaf litter is expected to increase permeation rate, and enhances contact between atmosphere and the biologically active soil layer (Cheng et al., 2013; Dong et al., 1998; Leitner et al., 2016). However, in high rainfall events, leaf litter can also store some precipitation, maintaining gas diffusivity within mineral soils compared to no litter treatment (Wang et al., 2013). In addition to regulating soil microclimate, fresh litter can also provide carbon sources, stimulating microbial growth and activity (S. Xu et al., 2013).

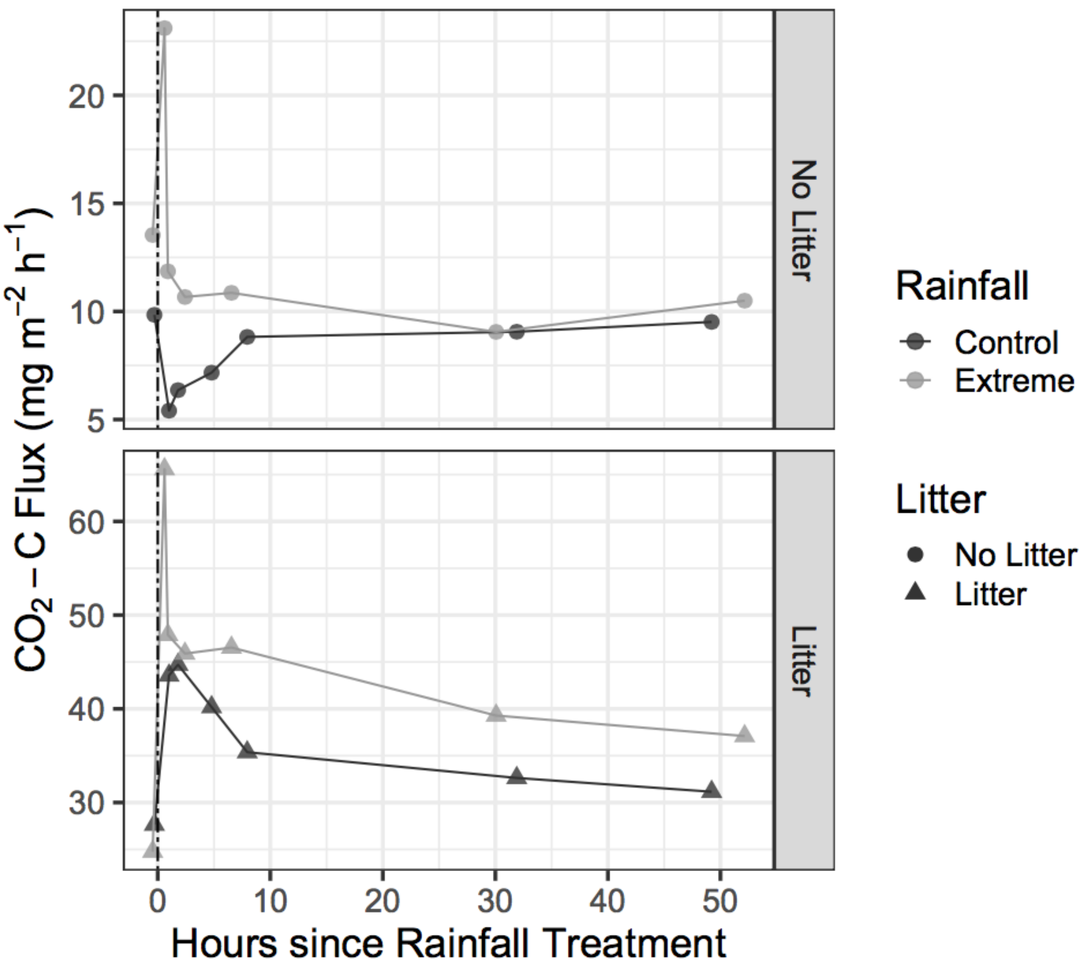

Figure 5-8 A typical temporal change of CO_2 flux after different rainfall treatments at week 13. Circles: litter treatment; triangles: no litter treatment. Vertical dashed line: rainfall treatments. Note the different scales on y axis

In the present study leaf litter had a significant effect in all of our response variables in all of the treatments. As expected, litter acted as a barrier for water to evaporate, resulting in higher soil moisture in those treatments. Mass loss of leaf litter cover over the course of 6 months decreased this effect especially in the control treatment (Figure 5-2); this is further supported by the GWC results that described soil moisture conditions throughout the entire column (Figure 5-3). According to our hypothesis, leaf litter increased CO_2 flux; however contrary to our expectations, CH_4 uptake also increased. These results partially support our first hypothesis. Rainfall intensity and frequency affected CO_2 production only on the short term, supporting our second hypothesis. The CH_4 fluxes were not affected by precipitation thus our third hypothesis has to be rejected.

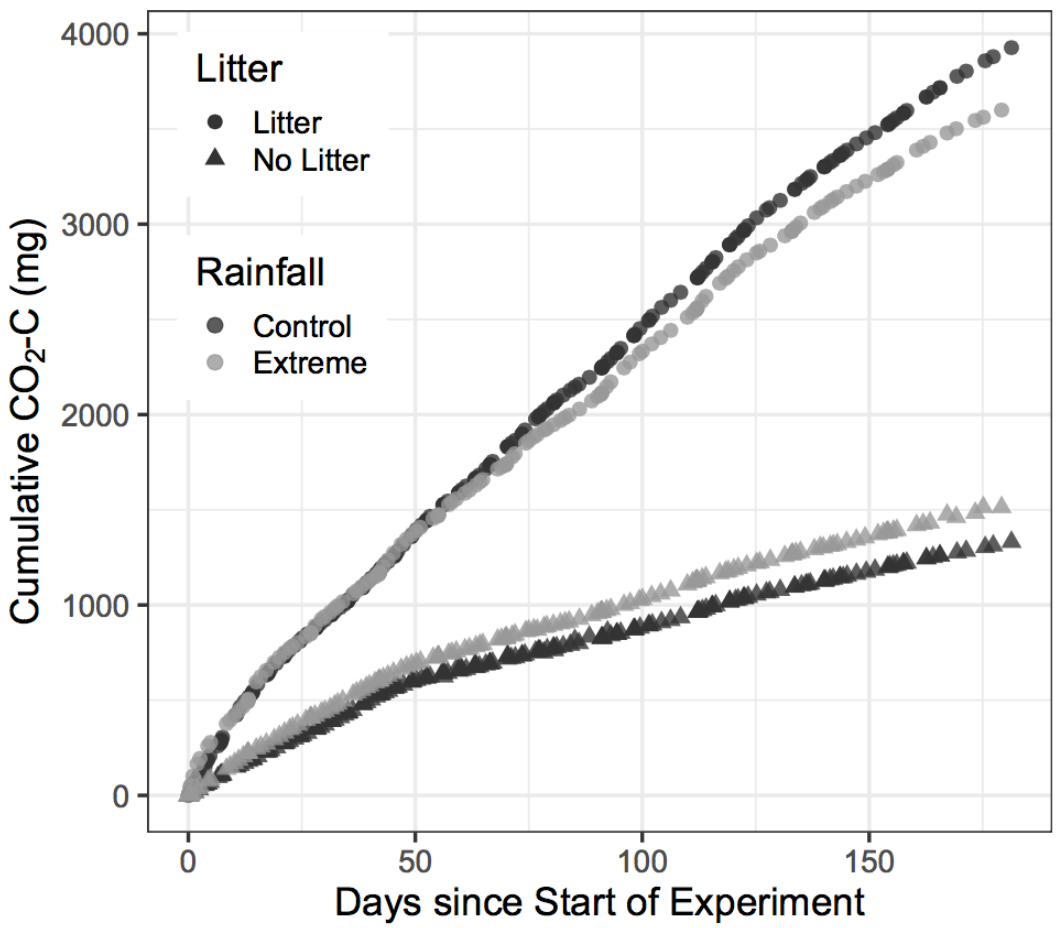

Figure 5-9 Cumulative CO2 in different rainfall treatments. Each point is the mean of three columns of the same treatment

5.4.1 Leaf litter increased CH_4 uptake

The present study is the first to simultaneously explore the effects of litter and precipitation variability on forest soil CH_4 uptake. Most laboratory studies (Blankinship et al., 2010; Bowden et al., 1998; Kravchenko & Sukhacheva, 2017) on CH_4 uptake were conducted in small closed jars with significantly less soil (5 to 170 g), so it is difficult to make comparisons between those results and ours. The mean values of CH_4 uptake in our experiment were much lower than the values of other temperate forest upland soils in the field (Blankinship et al., 2010; W. Borken et al., 2006; Fest et al., 2017; Muhr et al., 2008; Pitz et al., 2018). However, considering the disturbance of microbial community when homogenizing the soils and high soil moisture values throughout our experiment, our fluxes were reasonable.

Soil moisture is an important driver of CH_4 uptake capacity, which can directly affect methanotroph activity, or indirect influence gas diffusion rates and oxygen availability (B. K. Singh et al., 2010). The effect of changing precipitation patterns on methane fluxes may have global significance as shown recently by Ni and Groffman (2018). They reported a decrease in CH_4 uptake by an average of 77% from 1988 to 2015 in forest soils located from 0 to 60 degrees N latitude due to increase of precipitation and soil hydrological flux. However, in our experiment, CH_4 uptake rates showed no moisture dependence except a negative relationship in CNL treatment (Table 5-5) due to high soil moisture. In all but six CH_4 flux measurements, the corresponding VWC values were over 30%. The optimum moisture range for CH_4 oxidation varies between 10% and 25% (VWC) for different soils (Jay Shankar Singh, 2013). In a laboratory experiment, adjusting soil water contents between 25% and 75% of pore volume in forest soils did not affect the rate of CH_4 consumption (Nesbit & Breitenbeck, 1992). Our findings cannot be simply transferred to field conditions. In the field other important pathways of water, such as transpiration and runoff exist, which can lead to greater variability of soil moisture on multiple temporal scales.

Contrary to our expectation, CH_4 uptake was higher in the presence of leaf litter. Apart from soil moisture, gas diffusivity can be affected by compaction, which might be higher in the absence of leaf litter. We measured the height of surface horizon and subsurface horizon soils at the beginning and end of the experiment. Soil columns shrank less than 1%, which is insufficient to explain the approximately twofold CH_4 uptake difference between litter and no litter treatments.

Another important factor that could influence CH_4 uptake capacity in soils is the different methanotroph microbial activity in litter and no litter treatments. First, leaf litter provided additional surface area exposed to atmospheric concentrations of oxygen and CH_4 for methanotrophs to colonize. High CH_4 oxidation rates have been shown to occur in soils with a thick O layer (Prieme & Christensen, 1997; Wolf, Flessa, & Veldkamp, 2012). Second, methanotroph activity may be regulated by other factors, such as the availability of nitrogen (Malghani et al., 2016; Wolf et al., 2012), or other carbon sources (West & Schmidt, 1998, 1999). Under repeated drying and wetting, high quality litter used in our experiment could provide water-soluble carbon and nitrogen for methanotrophs to grow and thrive. Furthermore, positive correlation between CH_4 uptake and CO_2 efflux (Figure 5-7), and the decreasing trend of CH_4 uptake throughout the experiment (Figure 5-4) suggest that higher CH_4 uptake occurred in soils when conditions were generally more favorable for microbial activity. Third, previous studies (W. Borken et al., 2006; Cheng et al., 2013; Dong et al., 1998; Wang et al., 2013) focusing on litter effect on CH_4 fluxes, have been conducted with coniferous species. Different tree species have varied effects on CH_4 sinks. In general, soils under hardwood species (aspen, beech, birch, oak) have been shown to consume more CH_4 than soils under coniferous species (larch, pine, spruce) (Menyailo & Hungate, 2003; Nazaries et al., 2013; Shukla et al., 2013) due to differences in methanotroph communities (Degelmann, Borken, Drake, & Kolb, 2010; Menyailo et al., 2008). leaf structures and litter quality (W. Borken & Beese, 2006).

5.4.2 Extreme rainfall triggered a bigger pulse of CO_2 but had no effect on cumulative flux

Depending on the initial soil conditions, intense rainfall events can result in a CO_2 pulse or respiration can be depressed. CO_2 pulse is a well-known phenomenon and has frequently been reported both in laboratory experiments (X. Z. Liu, Wan, Su, Hui, & Luo, 2002; Miller et al., 2005; Muhr et al., 2008; X. Wu et al., 2010) and field observations (W. Borken et al., 2003; Lee et al., 2004; X. Z. Liu et al., 2002; Wang, Wang, Wang, Guo, & Bao, 2012; X. Xu & Luo, 2012). A combination of physical and biological processes, such as replacement of air by water in the soil (Huxman et al., 2004; X. Z. Liu et al., 2002), wetting of leaves (Lee et al., 2004) and enhanced mineralization of soil organic matter (Werner Borken & Matzner, 2009; Miller et al., 2005) and litter carbon (Cisneros-Dozal et al., 2007; Wang et al., 2012) are the underlying mechanisms leading to CO_2 pulses. If the soils are already wet, additional precipitation can fill the remaining pore spaces and cause anoxic conditions thus decreasing biological activity (Suseela et al., 2012; Wang et al., 2012). This is the case in our CNL treatment (Figure 5-5) in which high soil moisture content was maintained especially after the first few weeks (Figure 5-2). In ENL, physical displacement is the main mechanism because the flux quickly dropped, even below pretreatment levels, and then gradually recovered (Figure 5-8). Litter treatments always exhibited a pulse of CO_2, indicating stimulated microbial activity after rainfall. In addition to immediate mineralization of litter and soil carbon, priming effect may also occur (Yakov Kuzyakov, 2010). During rainfall event, labile litter carbon was transported to the soil, leading to accelerated soil organic matter decomposition and thus increased CO_2 flux. This effect would be important especially at the beginning of experiment when litter contains more easily degradable water soluble compounds (Bjorn Berg, 2000). In this experiment we used labeled (^{13}C, ^{15}N) tulip poplar leaves and stable isotope analysis of the respired CO_2 clearly showed a priming effect (Yang et al., submitted).

In rainfall manipulation experiments the size of the CO_2 pulse has been shown to be positively related to the amount (W. Borken et al., 2003; Werner Borken & Matzner, 2009), and intensity (X. Xu & Luo, 2012), and negatively related to the frequency (L. Yan et al., 2014) of water applied. In our experiment high intensity rainfall additions and longer periods of drier conditions lead to higher pulses in the extreme treatment. Additionally, initial moisture conditions and the change in VWC during rain events clearly drove the magnitude of the pulse, but the sensitivity of the system is fundamentally different in the presence of leaf litter (Figure 5-5, Figure 5-6). Compared to mineral soil, the change of water conditions of leaf litter on CO_2 flux is likely to be more significant (Wang et al., 2012). In extreme conditions leaf litter stayed dry for longer periods of time in between rain events, but on the short-term leaves stayed moist longer after intense rain, keeping the pulse longer (Figure 5-8) (Cisneros-Dozal et al., 2007; Wang et al., 2012). Although the short-term dynamics of CO_2 between the rainfall patterns were significantly different, the total carbon loss as CO_2 was similar during the experimental period both with and without leaf litter. However, leaf litter within the same rainfall treatment approximately doubled cumulative CO_2 release, which is in consistent with other studies that leaf litter increased CO_2 efflux (McIntyre, Adams, Ford, & Grierson, 2009; Miller et al., 2005; Muhr et al., 2008).

Obviously, field conditions are more complex: intense rainfall events can lead to runoff, rapid infiltration and leaching, reducing the storage of water compared with small, long-lasting rainfall (Werner Borken & Matzner, 2009). Plant roots, which were absent in our experiment, may response differently to rainfall events depending on vegetation type and initial conditions (Li et al., 2018). Total rainfall amount also varies annually.

5.4.3 Implications of litter cover on temperate forest carbon fluxes

The effects of litter cover on both CH_4 uptake and CO_2 production point to the importance of forest floor in regulating carbon fluxes between the soil and atmosphere. In temperate deciduous forests leaf litter thickness varies seasonally with the largest input in the fall, and gradually decrease throughout the year. The rate of mass loss depends on local climate, litter type, and litter feeding fauna. In some situations, litter can completely disappear by early summer, leaving most of the soil surface exposed. This is the case with high abundance of earthworms, especially when the forest is dominated by trees producing high quality, palatable litter. In North America, colonization and establishment of European and Asian earthworm species resulted in loss of organic layer and greater temporal fluctuation of litter layer thickness and composition in many forests (Bohlen et al., 2004) including our study site in Maryland (Szlavecz et al., 2018; Szlavecz et al., 2011). As our experiments also indicated, elimination of litter layer could alter both CH_4 uptake and CO_2 production. Additionally, earthworms can either compact or decompact mineral soil (Blouin et al., 2013), depending on the initial soil conditions and earthworm species composition. The burrowing activity earthworms can lead to altered soil porosity, indirectly affecting gas exchange at the soil-atmosphere interface. To fully understand the response of forest soils to altered precipitation patterns, leaf litter dynamics and role of other biotic and abiotic factors in gas diffusion need to be taken into consideration.

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6. Conclusions

My projects investigated the effect of changing rainfall patterns, including increased rainfall amount and increased frequency of extreme events on carbon cycling in temperate forest soils.

In temperate forests, where soil moisture is not limiting, quadratic relationship was found between soil respiration and soil moisture. After a threshold moisture, soil respiration decreased due to low availability of oxygen. Increasing rainfall will enhance soil respiration only when soil moisture is below the threshold. The non-linear relationship should be implemented in models to better predict soil respiration in projected climate scenarios.

I also conducted the first laboratory and field experiment to explore how increase of extreme rainfall events, but not the total amount, would affect partitioning of litter carbon to three pathways: carbon dioxide (CO_2), dissolved organic carbon (DOC), and soil organic carbon (SOC), during decomposition. With ^{13}C labeled tulip poplar leaves, our experiments clearly showed rainfall effect on the relative importance of litter carbon fates: an increase in extreme rainfall events would transport more litter derived carbon to greater depth, where the labile carbon could interact with minerals to form SOC. Further experiments should examine the detailed chemical nature as well as the stability of newly formed SOC. In addition to rainfall intensity, timing of extreme rainfall events and initial soil moisture also affected how deep litter carbon had been translocated in the soil. Our experiments provide evidence that in addition to litter quality (C:N ratio, lignin concentration) (M. F. Cotrufo et al., 2013), rainfall patterns can also affect litter carbon portioning. If the phenomena we observed in our experiments could apply generally to temperate forests, the enhanced litter carbon transport with increase of extreme rainfall events would significantly influence soil carbon cycling and potentially provide a negative feedback to atmospheric CO_2 levels in the short term. Although we detected ^{13}C signatures at all depths, with the exception of surface soil under extreme rainfall, we did not observe a change in soil carbon content with litter addition. Most likely, our experiment was short to detect potential changes in the total carbon content. Long-term rainfall manipulation experiments and a detailed analysis of soil organic matter fractions may reveal differences in the absolute and relative amounts of the diverse forms of SOM as a result of changing precipitation.

The significant effect of litter layer on CO_2 production and CH_4 uptake points to the importance of forest floor in regulating carbon fluxes between the soil and atmosphere. When soil moisture is high, which may be the case in temperate forests in Northeastern United States in the future due to increase of rainfall amount (Hayhoe et al., 2006), leaf litter mineralization can still induce a CO_2 pulse after rainfall events. In temperate deciduous forests leaf litter thickness varies seasonally, with the largest input in fall and decreases afterwards. Litter layer can completely disappear by the early summer with high abundance of earthworms. This is the case at our study site: invasive European and Asian earthworm species resulted in loss of litter and greater temporal fluctuation of litter thickness (Szlavecz et al., 2018). Apart from earthworm effect, both litter quality and quantity are expected to change in the future with global and regional changes, including increased CO_2 level, increased temperature, altered rainfall patterns, and nitrogen deposition (Condit et al., 1996; McMahon et al., 2010). The change of litter cover needs to be taken into consideration when modelling annual CO_2 and CH_4 fluxes.

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Yang, X., Szlavecz, K., Chang, C., Pitz, S. *Soil respiration under different soil moisture conditions in a temperate forest in Maryland, USA*. Mid-InfraRed Technologies for Health and the Environment (MIRTHE) 9th Year Site Visit, Princeton University, November 3rd, 2015

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Publications

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Yang, X., Szlavecz K, K., Pitz, S., Langley, A. Extreme rainfall events transported more litter carbon to deeper horizon during decomposition (in preparation).

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Honors and Awards

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